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METHOD OF ADMINISTERING AN ANTIBODY

BACKGROUND OF THE INVENTION

Integrin receptors are important for regulating both lymphocyte recirculation and recruitment to sites of inflammation (Carlos, T.M. and Harlan, J.M., *Blood, 84*:2068-2101 (1994)). The human α4β7 integrin has several ligands, one of which is the mucosal vascular addressin MAdCAM-1 (Berlin, C., *et al.*, *Cell 74*:185-195 (1993); Erle, D.J., *et al.*, *J. Immunol. 153*:517-528 (1994)) expressed on high endothelial venules in mesenteric lymph nodes and Peyer's patches (Streeter, P.R., *et al.*, *Nature 331*:41-46 (1988)). As such, the α4β7 integrin acts as a homing receptor that mediates lymphocyte migration to intestinal mucosal lymphoid tissue (Schweighoffer, T., *et al.*, *J. Immunol. 151*:717-729 (1993)). In addition, the α4β7 integrin interacts with fibronectin and vascular cell adhesion molecule-1 (VCAM-1).

Inflammatory bowel disease (IBD), such as ulcerative colitis and Crohn's disease, for example, can be a debilitating and progressive disease involving inflammation of the gastrointestinal tract. Affecting an estimated two million people in the United States alone, symptoms include abdominal pain, cramping, diarrhea and rectal bleeding. IBD treatments have included anti-inflammatory drugs (such as,

corticosteroids and sulfasalazine), immunosuppressive drugs (such as, 6-mercaptopurine, cyclosporine and azathioprine) and surgery (such as, colectomy). Podolsky, *New Engl. J. Med., 325:*928-937 (1991) and Podolsky, *New Engl. J. Med., 325*:1008-1016 (1991). However, such therapeutic agents have not been effective in maintaining remission of IBD.

Antibodies against human α4β7 integrin, such as murine monoclonal antibody (mAb Act-1), interfere with α4β7 integrin binding to mucosal addressin cell adhesion molecule-1 (MAdCAM-1) present on high endothelial venules in mucosal lymph nodes. Act-1 was originally isolated by Lazarovits, A.I., *et al., J. Immunol. 133*:1857-1862 (1984), from mice immunized with human tetanus toxoid-specific T lymphocytes and was reported to be a mouse IgG1/κ antibody. More recent analysis of the antibody by Schweighoffer, T., *et al., J. Immunol. 151*:717-729 (1993) demonstrated that it can bind to a subset of human memory CD4+ T lymphocytes which selectively express the α4β7 integrin. However, a serious problem with using murine antibodies for therapeutic applications in humans is that they are highly immunogenic in humans and quickly induce a human anti-murine antibody response (HAMA), which reduces the efficacy of the mouse antibody in patients and can prevent continued administration. The HAMA response results in rapid clearance of the mouse antibody, severely limiting any therapeutic benefit.

Thus, a need exists for improved therapeutic approaches to inflammatory bowel diseases and other inflammatory disorders of mucosal tissues.

SUMMARY OF THE INVENTION

The invention relates to a method of administering an antibody (e.g., humanized antibody, human antibody). In one aspect the invention is a method of treating a human having a disease associated with leukocyte infiltration of mucosal tissues comprising administering to the human an effective amount of an immunoglobulin having binding specificity for α4β7 integrin. In preferred embodiments no more than about 8 mg

immunoglobulin per kg body weight is administered in a period of about one month. In particular embodiments, the immunoglobulin can include one or more complementarity determining regions (CDRs) having the amino acid sequence of a CDR of murine Act-1 mAb. LDP-02 is a preferred antibody for administration. The immunoglobulin can be administered in multiple doses and the interval between doses can be at least 1 day or longer. In particular embodiments, the interval between doses can be at least about 7, 14 or 21 days or about one month. In one embodiment, the amount of immunoglobulin administered per dose can be an amount which is sufficient to achieve about 50% or greater saturation of $\alpha4\beta7$ binding sites on circulating lymphocytes and/or about 50% or greater inhibition of $\alpha4\beta7$ integrin expression on the surface of circulating lymphocytes for a period of at least about 10 days following administration of the dose. In another embodiment, the amount of immunoglobulin administered per dose can be an amount which is sufficient to achieve and maintain a serum concentration of said immunoglobulin of at least about 1 μg/mL for a period of about 10 days following administration of the dose.

The immunoglobulin can be administered alone or together with one or more other agents to treat a disease associated with leukocyte infiltration of mucosal tissues. For example, the immunoglobulin can be administered with steroids, immunosuppressive agents, non-steroidal anti-inflammatory agents or immunomodulators. In a preferred embodiment immunoglobulin is administered to treat a human having an inflammatory bowel disease, such as Crohn's disease or ulcerative colitis.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is an illustration of the nucleotide sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of the mouse (*Mus musculus*) Act-1 light chain variable region joined to the mouse Act-1 light chain signal peptide sequence.

FIG. 2 is an illustration of the nucleotide sequence (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of the mouse Act-1 antibody heavy chain variable region. The nucleotide sequence of the variable region is joined to a nucleotide sequence which encodes a deduced mouse Act-1 heavy chain signal peptide sequence, to yield a composite sequence. (The identity of the primer which amplified the heavy chain region was deduced from the degenerate sequence, and an amino acid sequence for the signal peptide was derived from the primer, downstream sequence and sequences of other signal peptides. The signal peptide shown may not be identical to that of the Act-1 hybridoma.)

FIG. 3 is an illustration of the nucleotide sequence (SEQ ID NO:5) and amino acid sequence (SEQ ID NO:6) of a portion of the heavy chain of a humanized Act-1 antibody (LDP-02) with a heavy chain signal peptide.

FIG. 4 is an illustration of the nucleotide sequence (SEQ ID NO:7) and amino acid sequence (SEQ ID NO:8) of a portion of the light chain of a humanized Act-1 antibody (LDP-02) with a light chain signal peptide.

FIG. 5 is an illustration of the amino acid sequence of the light chain complementarity determining regions (CDR1, SEQ ID NO: 9; CDR2, SEQ ID NO:10; CDR3, SEQ ID NO:11) and heavy chain complementarity determining regions (CDR1, SEQ ID NO: 12; CDR2, SEQ ID NO:13; CDR3, SEQ ID NO:14) of murine antibody Act-1 and LDP-02.

FIG. 6 is a graph showing mean serum LDP-02 levels (μg/ml) in healthy men over time following a single administration of LDP-02. Mean serum LDP-02 levels became negligible by day 36 following administration of 0.15 mg/kg by intravenous (IV)(-◇-) or subcutaneous (SC)(-■-) injection and following administration of 0.5 mg/kg by intravenous injection (-▲-). However serum LDP-02 was still measurable beyond day 36 following administration of 1.5 mg/kg (-x-) or 2.5 mg/kg (-*-) by intravenous injection.

FIG. 7 is a graph showing persistent loss of α4β7 signal (detected with Act-1 mAb) following administration of LDP-02. About 90% of α4β7 signal was rapidly lost (MESF ≈10%) after administration of LDP-02 and persisted following administration of all LDP-02 doses. Between about day 7 and day 22, α4β7 signal started to return to baseline for the 0.15 mg/kg IV dose group (-♦-) and for the 0.15 mg/kg SC dose group (-■-). Between day 22 and day 36, α4β7 signal started to return to baseline for the 0.5 mg/kg IV (-▲-) dose group. At the higher doses of LDP-02 studied (1.5 mg/kg (-x-) and 2.5 mg/kg (-*-)), loss of α4β7 signal persisted for longer than 36 days following single IV doses. For the 2.5 mg/kg dose group (-*-), loss of α4β7 signal persisted up to about Day 70 (data provided in Appendix to Study L297-007). MESF: mean equivalent soluble fluorescence.

FIG. 8 is a graph showing mean serum LDP-02 levels (μg/ml) in patients with ulcerative colitis over time following a single administration of LDP-02. Mean serum LDP-02 levels rose rapidly following administration of LDP-02. The concentration of serum LDP-02 fell to below 1.0 μg/mL in patients administered LDP-02 at 0.15 mg/kg by intravenous (-▲-) or subcutanious (-●-) injection by 10 days following dosing. However, serum LDP-02 concentrations remained above 1.0 μg/mL for about 20 days following administration of 0.5 mg/kg by intravenous injection (-■-). The serum concentration of LDP-02 remained above 1 μg/mL for about 60 days following administration of 2.0 mg/kg by intravenous injection (-▼-).

FIG. 9 is a graph showing persistent loss of α4β7 signal (detected with Act-1 mAb) following administration of LDP-02. About 90% of α4β7 signal was rapidly lost (MESF ≈10%) after administration of LDP-02 and the duration of signal loss was dependent upon dose. Starting at about Day 10, α4β7 signal started to return to baseline for the group administered 0.15 mg/kg of LDP-02 by IV (-■-) or SC (-◈-) injection. However, α4β7 signal started to return to baseline between day 30 and day 60 for the group administered 0.5 mg/kg (-▲-) intravenously, and after day 60 for the group

administered 2.0 mg/kg (-x-) intravenously (data provided in Appendix to Study L297-006). MESF: mean equivalent soluble fluorescence.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of administering an antibody (immunoglobulin) to a subject. In one aspect, the antibody to be administered is a human or humanized antibody having binding specificity for α4β7 integrin (e.g., ,mammalian α4β7 (e.g., human (*Homo sapiens*) α4β7). Preferably, the human or humanized immunoglobulins can bind α4β7 integrin with an affinity of at least about 10^7 M^{-1}, preferably at least about 10^8 M^{-1}, and more preferably at least about 10^9 M^{-1}. In one embodiment, the humanized immunoglobulin includes an antigen binding region of nonhuman origin which binds α4β7 integrin and a constant region derived from a human constant region. In another embodiment, the humanized immunoglobulin which binds α4β7 integrin comprises a complementarity determining region of nonhuman origin and a variable framework region of human origin, and if desired, a constant region of human origin. For example, the humanized immunoglobulin can comprise a heavy chain and a light chain, wherein the light chain comprises a complementarity determining region derived from an antibody of nonhuman origin which binds α4β7 integrin and a framework region derived from a light chain of human origin, and the heavy chain comprises a complementarity determining region derived from an antibody of nonhuman origin which binds α4β7 integrin and a framework region derived from a heavy chain of human origin.

Naturally occurring immunoglobulins have a common core structure in which two identical light chains (about 24 kD) and two identical heavy chains (about 55 or 70 kD) form a tetramer. The amino-terminal portion of each chain is known as the variable (V) region and can be distinguished from the more conserved constant (C) regions of the remainder of each chain. Within the variable region of the light chain is a C-terminal portion known as the J region. Within the variable region of the heavy chain,

there is a D region in addition to the J region. Most of the amino acid sequence variation in immunoglobulins is confined to three separate locations in the V regions known as hypervariable regions or complementarity determining regions (CDRs) which are directly involved in antigen binding. Proceeding from the amino-terminus, these regions are designated CDR1, CDR2 and CDR3, respectively. The CDRs are held in place by more conserved framework regions (FRs). Proceeding from the amino-terminus, these regions are designated FR1, FR2, FR3, and FR4, respectively. The locations of CDR and FR regions and a numbering system have been defined by Kabat *et al.* (Kabat, E.A. *et al., Sequences of Proteins of Immunological Interest,* Fifth Edition, U.S. Department of Health and Human Services, U.S. Government Printing Office (1991)).

Human immunoglobulins can be divided into classes and subclasses, depending on the isotype of the heavy chain. The classes include IgG, IgM, IgA, IgD and IgE, in which the heavy chains are of the gamma (γ), mu (μ), alpha (α), delta (δ) or epsilon (ϵ) type, respectively. Subclasses include IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2, in which the heavy chains are of the $\gamma 1$, $\gamma 2$, $\gamma 3$, $\gamma 4$, $\alpha 1$ and $\alpha 2$ type, respectively. Human immunoglobulin molecules of a selected class or subclass may contain either a kappa (κ) or lambda (λ) light chain. See e.g., *Cellular and Molecular Immunology*, Wonsiewicz, M.J., Ed., Chapter 45, pp. 41-50, W. B. Saunders Co, Philadelphia, PA (1991); Nisonoff, A., *Introduction to Molecular Immunology*, 2nd Ed., Chapter 4, pp. 45-65, Sinauer Associates, Inc., Sunderland, MA (1984).

The term "immunoglobulin" as used herein includes whole antibodies and biologically functional fragments thereof. Such biologically functional fragments retain at least one antigen binding function of a corresponding full-length antibody (e.g., specificity for $\alpha 4\beta 7$ of Act-1 antibody), and preferably, retain the ability to inhibit the interaction of $\alpha 4\beta 7$ with one or more of its ligands (e.g., MAdCAM-1, fibronectin). In a particularly preferred embodiment, biologically functional fragments can inhibit binding of $\alpha 4\beta 7$ to the mucosal addressin (MAdCAM-1). Examples of biologically

functional antibody fragments which can be administered as described herein include fragments capable of binding to an α4β7 integrin, such as single chain antibodies, Fv, Fab, Fab' and $F(ab')_2$ fragments. Such fragments can be produced by enzymatic cleavage or by recombinant techniques. For example, papain or pepsin cleavage can generate Fab or $F(ab')_2$ fragments, respectively. Other proteases with the requisite substrate specificity can also be used to generate Fab, $F(ab')_2$ or other antigen-binding fragments. Antibodies can also be produced in a variety of truncated forms using antibody genes in which one or more stop codons have been introduced upstream of the natural stop site. For example, a chimeric gene encoding a $F(ab')_2$ heavy chain portion can be designed to include DNA sequences encoding the CH_1 domain and hinge region of the heavy chain.

The term "humanized immunoglobulin" as used herein refers to an immunoglobulin (antibody) comprising portions of immunoglobulins of different origin, wherein at least one portion is of human origin. For example, the humanized antibody can comprise portions derived from an immunoglobulin of nonhuman origin with the requisite specificity, such as a mouse, and from immunoglobulin sequences of human origin (e.g., chimeric immunoglobulin), joined together chemically by conventional techniques (e.g., synthetic) or prepared as a contiguous polypeptide using recombinant DNA technology (e.g., DNA encoding the protein portions of the chimeric antibody can be expressed to produce a contiguous polypeptide chain). Another example of a humanized immunoglobulin is an immunoglobulin containing one or more immunoglobulin chains comprising a CDR derived from an antibody of nonhuman origin and a framework region derived from a light and/or heavy chain of human origin (e.g., CDR-grafted antibodies with or without framework changes). Chimeric or CDR-grafted single chain antibodies are also encompassed by the term humanized immunoglobulin. See, e.g., Cabilly *et al.*, U.S. Patent No. 4,816,567; Cabilly *et al.*, European Patent No. 0,125,023 B1; Boss *et al.*, U.S. Patent No. 4,816,397; Boss *et al.*, European Patent No. 0,120,694 B1; Neuberger, M.S. *et al.*, WO 86/01533; Neuberger,

M.S. *et al.*, European Patent No. 0,194,276 B1; Winter, U.S. Patent No. 5,225,539; Winter, European Patent No. 0,239,400 B1; Queen *et al.*, European Patent No. 0 451 216 B1; Padlan, E.A. *et al.*, European Patent Application No. 0,519,596 A1. See also, Ladner *et al.,* U.S. Patent No. 4,946,778; Huston, U.S. Patent No. 5,476,786; and Bird, R.E. *et al., Science, 242:* 423-426 (1988)), regarding single chain antibodies. In particular embodiments, the humanized immunoglobulin can include an immunoglobulin chain (e.g., heavy chain) having a variable region of non-human origin (e.g., murine origin) and at least a portion of a human constant region (e.g, Cγ1), and an immunoglobulin chain (e.g., light chain) where at least one CDR is of non-human origin (e.g., murine origin) and the framework regions (FR1, FR2, FR3, FR4) and, optionally, the constant region (e.g., Cκ, Cλ) are of human origin.

The antigen binding region of the humanized immunoglobulin (the nonhuman portion) can be derived from an immunoglobulin of nonhuman origin (referred to as a donor immunoglobulin) having binding specificity for α4β7 integrin. For example, a suitable antigen binding region can be derived from the murine Act-1 monoclonal antibody (Lazarovits, A.I. *et al., J. Immunol., 133(4)*: 1857-1862 (1984)). Other sources include α4β7 integrin-specific antibodies obtained from nonhuman sources, such as rodent (e.g., mouse, rat), rabbit, pig goat or non-human primate (e.g., monkey). Other polyclonal or monoclonal antibodies, such as antibodies which bind to the same or similar epitope as the Act-1 antibody, or LDP-02, can be made (e.g., Kohler *et al.*, *Nature, 256*:495-497 (1975); Harlow *et al.*, 1988, Antibodies: A Laboratory Manual, (Cold Spring Harbor, NY); and Current Protocols in Molecular Biology, Vol. 2 (Supplement 27, Summer '94), Ausubel *et al.*, Eds. (John Wiley & Sons: New York, NY), Chapter 11 (1991)).

For example, antibodies can be raised against an appropriate immunogen in a suitable mammal (e.g., a mouse, rat, rabbit, sheep). Preparation of immunizing antigen, and polyclonal and monoclonal antibody production can be performed using any suitable technique. A variety of methods have been described (see e.g., Kohler *et al.*,

Nature, 256: 495-497 (1975) and *Eur. J. Immunol. 6:* 511-519 (1976); Milstein *et al., Nature 266: 550-552 (1977);* Koprowski *et al.,* U.S. Patent No. 4,172,124; Harlow, E. and D. Lane, 1988, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory: Cold Spring Harbor, NY); *Current Protocols In Molecular Biology*, Vol. 2 (Supplement 27, Summer '94), Ausubel, F.M. *et al.*, Eds., (John Wiley & Sons: New York, NY), Chapter 11, (1991)). For example, suitable immunizing agents include cells bearing α4β7, membrane fractions containing α4β7, immunogenic fragments of suitable immunogens include α4β7, a β7 peptide conjugated to a suitable carrier and the like. Antibody-producing cells (e.g., a lymphocyte) can be isolated from, for example, the lymph nodes or spleen of an immunized animal. The cells can then be fused to a suitable immortalized cell (e.g., a myeloma cell line (e.g., SP2/0, P3x63Ag8.653), thereby forming a hybridoma. Fused cells can be isolated employing selective culturing techniques. Cells which produce antibodies with the desired specificity can be selected using a suitable assay (e.g., ELISA). Other suitable methods of producing or isolating antibodies (human antibodies, non-human antibodies) of the requisite specificity can be used, including, for example, methods which select recombinant antibody from a library (e.g., a phage display library). Transgenic animals capable of producing a repertoire of human antibodies (e.g., Xenomouse (Abgenix, Fremont, CA) can be produced using suitable methods (see e.g., WO 98/24893 (Abgenix), published June 11, 1998; Kucherlapate, R. and Jakobovits, A., U.S. Patent No. 5,939,598; Jakobovits *et al., Proc. Natl. Acad. Sci. USA, 90*: 2551-2555 (1993); Jakobovits *et al., Nature, 362*: 255-258 (1993)). Additional methods for production of transgenic animals capable of producing a repertoire of human antibodies have been described (e.g., Lonberg *et al.*, U.S. Patent No. 5,545,806; Surani *et al.*, U.S. Patent No. 5,545,807; Lonberg *et al.*, WO97/13852).

In one embodiment, the antigen binding region of the humanized immunoglobulin comprises a CDR of nonhuman origin. In this embodiment, the humanized immunoglobulin having binding specificity for α4β7 integrin comprises at

least one CDR of nonhuman origin. For example, CDRs can be derived from the light and heavy chain variable regions of immunoglobulins of nonhuman origin, such that a humanized immunoglobulin includes substantially heavy chain CDR1, CDR2 and/or CDR3, and/or light chain CDR1, CDR2 and/or CDR3, from one or more immunoglobulins of nonhuman origin, and the resulting humanized immunoglobulin has binding specificity for α4β7 integrin. Preferably, all three CDRs of a selected chain are substantially the same as the CDRs of the corresponding chain of a donor, and more preferably, all six CDRs of the light and heavy chains are substantially the same as the CDRs of the corresponding donor chains. In a preferred embodiment, the one or more CDRs of nonhuman origin have the amino acid sequences of the CDRs of murine Act-1 Ab (SEQ ID Nos. 9-14).

The portion of the humanized immunoglobulin or immunoglobulin chain which is of human origin (the human portion) can be derived from any suitable human immunoglobulin or immunoglobulin chain. For example, a human constant region or portion thereof, if present, can be derived from the κ or λ light chains, and/or the γ (e.g., γ1, γ2, γ3, γ4), μ, α (e.g., α1, α2), δ or ϵ heavy chains of human antibodies, including allelic variants. A particular constant region (e.g., IgG1), variant or portions thereof can be selected in order to tailor effector function. For example, a mutated constant region (variant) can be incorporated into a fusion protein to minimize binding to Fc receptors and/or ability to fix complement (see e.g., Winter *et al.*, GB 2,209,757 B; Morrison *et al.*, WO 89/07142; Morgan *et al.*, WO 94/29351, December 22, 1994). LDP-02 contains a heavy chain constant region (human γ1 heavy chain constant region) that was modified to reduce binding to human Fcγ receptors. The LDP-02 Fc modification are at positions 235 and 237 (i.e., $Leu^{235} \rightarrow Ala^{235}$ and $Gly^{237} \rightarrow Ala^{237}$).

If present, human framework regions (e.g., of the light chain variable region) are preferably derived from a human antibody variable region having sequence similarity to the analogous region (e.g., light chain variable region) of the antigen binding region donor. Other sources of framework regions for portions of human origin of a

humanized immunoglobulin include human variable consensus sequences (see e.g., Kettleborough, C.A. *et al.*, *Protein Engineering 4*:773-783 (1991); Carter *et al.*, WO 94/04679, published March 3, 1994)). For example, the sequence of the antibody or variable region used to obtain the nonhuman portion can be compared to human sequences as described in Kabat, E.A., *et al., Sequences of Proteins of Immunological Interest,* Fifth Edition, U.S. Department of Health and Human Services, U.S. Government Printing Office (1991). In a particularly preferred embodiment, the framework regions of a humanized immunoglobulin chain are derived from a human variable region having at least about 65% overall sequence identity, and preferably at least about 70% overall sequence identity, with the variable region of the nonhuman donor antibody (e.g., mouse Act-1 antibody). A human portion can also be derived from a human antibody having at least about 65% sequence identity, and preferably at least about 70% sequence identity, within the particular portion (e.g., FR) being used, when compared to the equivalent portion (e.g., FR) of the nonhuman donor. Amino acid sequence identity can be determined using a suitable sequence alignment algorithm, such as the Lasergene system (DNASTAR, Inc., Madison, WI), using the default parameters.

In one embodiment, the humanized immunoglobulin comprises at least one of the framework regions (FR) derived from one or more chains of an antibody of human origin. Thus, the FR can include a FR1 and/or FR2 and/or FR3 and/or FR4 derived from one or more antibodies of human origin. Preferably, the human portion of a selected humanized chain includes FR1, FR2, FR3 and FR4 derived from a variable region of human origin (e.g., from a human immunoglobulin chain, from a human consensus sequence).

The immunoglobulin portions of nonhuman and human origin for use in preparing humanized antibodies can have sequences identical to immunoglobulins or immunoglobulin portions from which they are derived or to variants thereof. Such variants include mutants differing by the addition, deletion, or substitution of one or

more residues. As indicated above, the CDRs which are of nonhuman origin are substantially the same as in the nonhuman donor, and preferably are identical to the CDRs of the nonhuman donor. Changes in the framework region, such as those which substitute a residue of the framework region of human origin with a residue from the corresponding position of the donor, can be made. One or more mutations in the framework region can be made, including deletions, insertions and substitutions of one or more amino acids. For a selected humanized antibody or chain, suitable framework mutations can be designed. Preferably, the humanized immunoglobulins can bind $\alpha4\beta7$ integrin with an affinity similar to or better than that of the nonhuman donor. Variants can be produced by a variety of suitable methods, including mutagenesis of nonhuman donor or acceptor human chains.

Immunoglobulins (e.g., human and/or humanized immunoglobulins) having binding specificity for human $\alpha4\beta7$ integrin include immunoglobulins (including antigen-binding fragments) which can bind determinants (epitopes) of the $\alpha4$ chain (e.g., mAb HP1/2 (Pulido, et al., J Biol Chem 266:10241-10245 (1991), murine MAb 21.6 and humanized MAb 21.6 (Bendig *et al.*, U.S. Patent No. 5,840,299)) and/or the $\beta7$ chain of the $\alpha4\beta7$ heterodimer. For example, in particular embodiments, the human or humanized immunoglobulin can specifically or selectively bind a determinant of the $\alpha4\beta7$ complex, but not bind determinants (epitopes) on the $\alpha4$ chain or the $\beta7$ chain. In one embodiment, the human or humanized immunoglobulin can have binding specificity for a combinatorial epitope on the $\alpha4\beta7$ heterodimer. Such an immunoglobulin can bind $\alpha4\beta7$ and not bind $\alpha4\beta1$, for example. Antibodies which have binding specificity for the $\alpha4\beta7$ complex include, murine Act-1 antibody and a humanized Act-1 referred to as LDP-02 (see, WO 98/06248 by LeukoSite, Inc., published February 19, 1998 and U.S. Application No. 08/700,737, filed August 15, 1996, the entire teachings of which are both incorporated herein by reference). In a preferred embodiment, the humanized immunoglobulin has at least one function characteristic of murine Act-1 antibody, such as binding function (e.g., having

specificity for α4β7 integrin, having the same or similar epitopic specificity), and/or inhibitory function (e.g., the ability to inhibit α4β7-dependent adhesion *in vitro* and/or *in vivo*, such as the ability to inhibit α4β7 integrin binding to MAdCAM-1 *in vitro* and/or *in vivo*, or the ability to inhibit the binding of a cell bearing α4β7 integrin to a ligand thereof (e.g., a cell bearing MAdCAM-1)). Thus, preferred humanized immunoglobulins can have the binding specificity of the murine Act-1 antibody, the epitopic specificity of murine Act-1 antibody (e.g., can compete with murine Act-1, a chimeric Act-1 antibody, or humanized Act-1 (e.g., LDP-02) for binding to α4β7 (e.g., on a cell bearing α4β7 integrin)), and/or inhibitory function. A particularly preferred humanized Ab for administration in accordance with the method is LDP-02.

The binding function of a human or humanized immunoglobulin having binding specificity for α4β7 integrin can be detected by standard immunological methods, for example using assays which monitor formation of a complex between humanized immunoglobulin and α4β7 integrin (e.g., a membrane fraction comprising α4β7 integrin, on a cell bearing α4β7 integrin, such as a human lymphocyte (e.g., a lymphocyte of the CD4+$\alpha4^{hi}$,$\beta1^{lo}$ subset), human lymphocyte cell line or recombinant host cell comprising nucleic acid encoding α4 and/or β7 which expresses α4β7 integrin). Binding and/or adhesion assays or other suitable methods can also be used in procedures for the identification and/or isolation of immunoglobulins (e.g., human and/or humanized immunoglobulins) (e.g., from a library) with the requisite specificity (e.g., an assay which monitors adhesion between a cell bearing an α4β7 integrin and a ligand thereof (e.g., a second cell expressing MAdCAM, an immobilized MAdCAM fusion protein (e.g., MAdCAM-Ig chimera)), or other suitable methods.

The immunoglobulin portions of nonhuman and human origin for use in preparing humanized immunoglobulins include light chains, heavy chains and portions of light and heavy chains. These immunoglobulin portions can be obtained or derived from immunoglobulins (e.g., by *de novo* synthesis of a portion), or nucleic acids encoding an immunoglobulin or chain thereof having the desired property (e.g., binds

$\alpha4\beta7$ integrin, sequence similarity) can be produced and expressed. Humanized immunoglobulins comprising the desired portions (e.g., antigen binding region, CDR, FR, constant region) of human and nonhuman origin can be produced using synthetic and/or recombinant nucleic acids to prepare genes (e.g., cDNA) encoding the desired humanized chain. To prepare a portion of a chain, one or more stop codons can be introduced at the desired position. For example, nucleic acid (e.g., DNA) sequences coding for newly designed humanized variable regions can be constructed using PCR mutagenesis methods to alter existing DNA sequences (see e.g., Kamman, M., *et al.*, *Nucl. Acids Res. 17*:5404 (1989)). PCR primers coding for the new CDRs can be hybridized to a DNA template of a previously humanized variable region which is based on the same, or a very similar, human variable region (Sato, K., *et al.*, *Cancer Research 53*:851-856 (1993)). If a similar DNA sequence is not available for use as a template, a nucleic acid comprising a sequence encoding a variable region sequence can be constructed from synthetic oligonucleotides (see e.g., Kolbinger, F., *Protein Engineering 8*:971-980 (1993)). A sequence encoding a signal peptide can also be incorporated into the nucleic acid (e.g., on synthesis, upon insertion into a vector). If the natural signal peptide sequence is unavailable, a signal peptide sequence from another antibody can be used (see, e.g., Kettleborough, C.A., *Protein Engineering 4*:773-783 (1991)). Using these methods, methods described herein or other suitable methods, variants can be readily produced. In one embodiment, cloned variable regions (e.g., of LDP-02) can be mutagenized, and sequences encoding variants with the desired specificity can be selected (e.g., from a phage library; see e.g., Krebber *et al.*, U.S. 5,514,548; Hoogenboom *et al.*, WO 93/06213, published April 1, 1993)).

Human and/or humanized immunoglobulins can be administered (e.g., to a human) for therapeutic and/or diagnostic purposes in accordance with the method of the invention. For example, an effective amount of a human and/or humanized immunoglobulins having binding specificity for $\alpha4\beta7$ integrin can be administered to a human to treat a disease associated with leukocyte infiltration of mucosal tissues (e.g.,

inflammatory bowel disease, such as Crohn's disease or ulcerative colitis). Treatment includes therapeutic or prophylactic treatment (e.g., maintenance therapy). According to the method, the disease can be prevented or delayed (e.g., delayed onset, prolonged remission or quiescence) or the severity of disease can be reduced in whole or in part.

In one embodiment, no more than about 8 mg of immunoglobulin per kg body weight is administered during a period of about 1 month. In additional embodiments, no more than about 7 or about 6 or about 5 or about 4 or about 3 or about 2 or about 1 mg of immunoglobulin per kg body weight is administered during a period of about 1 month. As used herein, the term "month" refers to a calendar month and encompasses periods of 28, 29, 30 and 31 days. When an antigen-binding fragment of a human or humanized immunoglobulin is to be administered, the amount which is administered during the period of about one month can be adjusted in accordance with the size of the fragment. For example, if the antigen-binding fragment is about half the size of the intact antibody by weight (e.g., measured in kDa), the amount administered during a period of about 1 month can be about 4 mg per kg body weight or less. The amount of immunoglobulin or antigen-binding fragment administered can be expressed as mg/kg body weight or using any other suitable units. For example, the amount of immunoglobulin or antigen-binding fragment administered can be expressed as moles of antigen binding sites per kg body weight. The number of moles of antigen-binding sites is dependent upon the size, quantity and valency of the immunoglobulin or fragment and can be readily determined. For example, IgG and $F(ab')_2$ fragments thereof are divalent and a dose which comprises 1 nanomole of IgG or $F(ab')_2$ fragment comprises 2 nanomoles of antigen-binding sites. The size of an antibody or antigen-binding fragment can be determined using any suitable method (e.g., gel filtration).

The human or humanized antibody or antigen-binding fragment can be administered in a single dose or in an initial dose followed by one or more subsequent doses. When multiple doses are desired, the interval between doses and the amount of immunoglobulin or antigen-binding fragment can be adjusted to achieve the desired

therapeutic and/or diagnostic effect. For example, each of the doses to be administered can independently comprise up to about 8 mg immunoglobulin or fragment per kg body weight. When a dose comprises about 8 mg immunoglobulin or fragment per kg body weight the minimum interval before a subsequent dose is administered is a period of about 1 month. Preferably, each dose independently comprises about 0.1 to about 8 mg or about 0.1 to about 5 mg immunoglobulin or fragment per kg body weight. More preferably, each dose independently comprises about 0.1 to about 2.5 mg immunoglobulin or fragment per kg body weight. Most preferably, each dose independently comprises about 0.15, about 0.5, about 1.0, about 1.5 or about 2.0 mg immunoglobulin or fragment per kg body weight.

The interval between any two doses (e.g., initial dose and first subsequent dose, first subsequent dose and second subsequent dose) can independently vary from a few seconds or minutes to about 120 days or more. For example, the initial dose can be administered and a first subsequent dose can be administered about 1 day later. Thereafter, second and third subsequent doses can be administered at intervals of about 1 month. Generally the minimum interval between doses is a period of at least about 1 day or at least about 7 days. In particular embodiments, the minimum interval between doses is a period of at least about 14 days, or at least about 21 days or at least about 1 month (e.g., 28, 29, 30, 31 days). In additional embodiments, the interval between doses can be at least about 40, about 50, about 60, about 70, about 80, about 90, about 100, about 110 or about 120 days.

The amount of human or humanized immunjoglobulin or antigen-binding fragments thereof administered in each dose can be an amount which is sufficient to produce a desired pharmacokinetic or pharmacodynamic effect. A variety of pharmacokinetic and pharmacodynamic parameters of human and/or humanized immunoglobulins or antigen-binding fragments thereof can be measured using suitable methods. For instance, pharmacodymanic parameters of antibodies and antigen-binding fragments (e.g., antigen saturation, antibody-induced inhibition of expression of antigen) can be measured using a suitable immunoassay. For example, as described

herein, $\alpha4\beta7$ signal (i.e., binding of labeled antibody to $\alpha4\beta7$) following administration of LDP-02 was measured by flow cytometry. The results of the assay revealed that administration of LDP-02 can result in saturation of $\alpha4\beta7$ and/or inhibition of expression of $\alpha4\beta7$ on the surface of circulating lymphocytes.

Accordingly, each dose to be administered can comprise an amount of immunoglobulin or fragment which is sufficient to achieve a) about 50% or greater saturation of $\alpha4\beta7$ integrin binding sites on circulating lymphocytes (e.g., CD8+ cells) and/or b) about 50% or greater inhibition of $\alpha4\beta7$ integrin expression on the cell surface of circulating lymphocytes for a period of at least about 10 days following administration of the dose. In other embodiments, each dose can comprise an amount of immunoglobulin or fragment which is sufficient to achieve and maintain a) about 60% or greater, about 70% or greater, about 80% or greater or about 85% or greater saturation of $\alpha4\beta7$ integrin binding sites on circulating lymphocytes and/or b) about 60% or greater, about 70% or greater, about 80% or greater or about 85% or greater inhibition of $\alpha4\beta7$ integrin expression on the cell surface of circulating lymphocytes for a period of at least about 10 days following administration of the dose.

In other particular embodiments, each dose can comprise an amount of immunoglobulin or fragment which is sufficient to achieve a desired degree of saturation of $\alpha4\beta7$ integrin binding sites on circulating lymphocytes (e.g., CD8+ cells) and/or inhibit expression of $\alpha4\beta7$ integrin on the cell surface of circulating lymphocytes to the desired degree for a period of at least about 14 days, at least about 20 days, at least about 25 days or at least about one month following administration of the dose. In additional embodiments, each dose can comprise an amount of immunoglobulin or fragment which is sufficient to achieve a desired degree of saturation of $\alpha4\beta7$ integrin binding sites on circulating lymphocytes (e.g., CD8+ cells) and/or inhibit expression of $\alpha4\beta7$ integrin on the cell surface of circulating lymphocytes to the desired degree for a period of at least about 40, about 50, about 60, about 70, about 80, about 90, about 100, about 110 or about 120 days.

Suitable assays for determining the dose of antibody required to achieve a desired serum concentration or to saturate and/or inhibit expression of a target antigen can be readily designed. For example, a flow cytometry based assay can be used to measure $\alpha4\beta7$ expression on the surface of cells isolated from a subject following administration of an immunoglobulin (e.g., human, humanized) which binds to $\alpha4\beta7$. In one embodiment, a murine antibody which binds human $\alpha4\beta7$ can be used. Preferably the murine antibody can bind to an epitope on $\alpha4\beta7$ which is distinct from the epitope bound by the human or humanized immunoglobulin and the binding of the murine antibody to $\alpha4\beta7$ is not inhibited (e.g., blocked) by the prior binding of the humanized immunoglobulin. Murine antibodies or other antibodies with these properties can be prepared and selected using the methods described herein or other suitable methods. The level of $\alpha4\beta7$ expression on circulating lymphocytes (e.g., CD8+ cells) isolated from a human can be measured or determined using each of the antibodies (i.e., immunoglobulin to be administered, murine antibody) by flow cytometry or other suitable methods. Then, the humanized antibody can be administered to the human, peripheral blood can be drawn at predetermined times following the administration and lymphocytes can be isolated (e.g., by density gradient centrifugation) for analysis. The peripheral blood lymphocytes (e.g., CD8+ cells) can be stained with each of the antibodies and the amount of $\alpha4\beta7$ detected by each antibody can be measured or detected by flow cytometry or other suitable methods. A decrease in the amount of $\alpha4\beta7$ integrin measured or determined using the human or humanized immunoglobulin is indicative of a) persistent integrin occupancy by the immunoglobulin (e.g., antigen saturation) and/or b) inhibition of $\alpha4\beta7$ expression on the surface of the lymphocytes (e.g., down modulation of $\alpha4\beta7$, shedding of $\alpha4\beta7$). A decrease in the amount of $\alpha4\beta7$ integrin measured or detected using the human or humanized immunoglobulin together with no change in the amount of $\alpha4\beta7$ integrin measured or determined using the murine antibody is indicative of persistent occupancy of $\alpha4\beta7$ (e.g., saturation) by the humanized immunoglobulin. A decrease in the amount of $\alpha4\beta7$

integrin measured or detected using the human or humanized immunoglobulin together with a decrease in the amount of $\alpha4\beta7$ integrin measured or detected using the murine antibody is indicative of inhibition of $\alpha4\beta7$ expression on the surface of circulating lymphocytes.

Pharmacokinetic parameters, such as the serum concentration of antibody over time following administration of said antibody can be measured using an immunoassay such as an ELISA or cell-based assay. For example, as described herein, the serum concentration of a humanized anti-$\alpha4\beta7$ immunoglobulin (LDP-02) at predetermined time points following a single administration of antibody (LDP-02) was measured using a cell-based assay. The results of the assay revealed that the serum concentration of LDP-02 can remain elevated (e.g., at or above 1 μg/ml) for a period of about 10 days or more following administration of the humanized antibody. The prolonged presence of LDP-02 in the serum can be indicative of superior efficacy as a result of persistent inhibition of $\alpha4\beta7$ function, for example persistent inhibition of $\alpha4\beta7$ mediated adhesion of leukocytes to MAdCAM.

Accordingly, each dose to be administered can comprise an amount of immunoglobulin or fragment which is sufficient to achieve and maintain a serum concentration of at least about 1 μg/mL for a period of at least about 10 days following administration of the dose. In particular embodiments, each dose can comprise amount of immunoglobulin or fragment which is sufficient to achieve and maintain a serum concentration of at least about 1 μg/mL for a period of at least about 14 days, at least about 20 days, at least about 25 days or at least about one month following administration of the dose. In additional embodiments, each dose can comprise amount of immunoglobulin or fragment which is sufficient to achieve and maintain a serum concentration of at least about 1 μg/mL for a period of at least about 40, about 50, about 60, about 70, about 80, about 90, about 100, about 110 or about 120 days.

As discussed herein, antigen-binding fragments of a human or humanized immunoglobulin can be substantially smaller and, therefore, bind more antigen ($\alpha4\beta7$)

per unit of protein (μg) than intact or native immunoglobulin. Accordingly, the serum concentration of an antigen-binding fragment of a human or humanized immunoglobulin which can be indicative of superior efficacy can be lower than 1 μg/mL. Thus, when administration of an antigen-binding fragment of a human or humanized immunoglobulin is desired, the dose can comprise an amount of antigen-binding fragment which is sufficient to achieve a serum concentration which is proportionate to 1 μg/mL for an intact immunoglobulin. For example, if the antigen-binding fragment is about half the size of the intact antibody by weight (e.g., measured in kDa), the dose can comprise an amount sufficient to achieve and maintain a serum concentration of about 0.5 μg/mL for a period of at least about 10 days. The desired serum concentration of immunoglobulin or antigen-binding fragment can be expressed as μg/mL or using any other suitable units. For example, the amount of immunoglobulin or antigen-binding fragment administered can be expressed as moles of antigen binding sites per volume of serum (e.g., M).

Human and humanized immunoglobulins can be administered in accordance with the present invention for *in vivo* diagnostic applications or to modulate α4β7 integrin function in therapeutic (including prophylactic) applications. For example, human and humanized immunoglobulins can be used to detect and/or measure the level of an α4β7 integrin in a subject. For example, a humanized immunoglobulin having binding specificity for α4β7 integrin can be administered to a human and antibody-α4β7 integrin complexes which are formed can be detected using suitable methods. For example, the humanized antibody can be labeled with, for example, radionuclides (^{125}I, ^{111}In, technetium-99m), an epitope label (tag), an affinity label (e.g., biotin, avidin), a spin label, an enzyme, a fluorescent group or a chemiluminescent group and suitable detection methods can be used. In an application of the method, humanized immunoglobulins can be used to analyze normal versus inflamed tissues (e.g., from a human) for α4β7 integrin reactivity and/or expression (e.g. radiologically) or to detect associations between IBD or other conditions and increased expression of α4β7 (e.g., in

affected tissues). The immunoglobulins described herein can be administered in accordance with the method of the invention for assessment of the presence of $\alpha4\beta7$ integrin in normal versus inflamed tissues, through which the presence of disease, disease progress and/or the efficacy of anti-$\alpha4\beta7$ integrin therapy in inflammatory disease can be assessed.

Human and humanized immunoglobulins (including antigen-binding fragments) can be administered to an individual to modulate (e.g., inhibit (reduce or prevent)) binding function and/or leukocyte (e.g., lymphocyte, monocyte) infiltration function of $\alpha4\beta7$ integrin. For example, human and humanized immunoglobulins which inhibit the binding of $\alpha4\beta7$ integrin to a ligand (i.e., one or more ligands) can be administered according to the method for the treatment of diseases associated with leukocyte (e.g., lymphocyte, monocyte) infiltration of tissues (including recruitment and/or accumulation of leukocytes in tissues), particularly of tissues which express the molecule MAdCAM. An effective amount of a human immunoglobulin or antigen-binding fragment thereof, or humanized immunoglobulin or antigen-binding fragment thereof (i.e., one or more immunoglobulins or fragments) is administered to an individual (e.g., a mammal, such as a human or other primate) in order to treat such a disease. For example, inflammatory diseases, including diseases which are associated with leukocyte infiltration of the gastrointestinal tract (including gut-associated endothelium), other mucosal tissues, or tissues expressing the molecule MAdCAM-1 (e.g., gut-associated tissues, such as venules of the lamina propria of the small and large intestine; and mammary gland (e.g., lactating mammary gland)), can be treated according to the present method. Similarly, an individual having a disease associated with leukocyte infiltration of tissues as a result of binding of leukocytes to cells (e.g., endothelial cells) expressing MAdCAM-1 can be treated according to the present invention.

In a particularly preferred embodiment, diseases which can be treated accordingly include inflammatory bowel disease (IBD), such as ulcerative colitis, Crohn's disease, ileitis, Celiac disease, nontropical Sprue, enteropathy associated with

seronegative arthropathies, microscopic or collagenous colitis, eosinophilic gastroenteritis, or pouchitis resulting after proctocolectomy, and ileoanal anastomosis.

Pancreatitis and insulin-dependent diabetes mellitus are other diseases which can be treated using the present method. It has been reported that MAdCAM-1 is expressed by some vessels in the exocrine pancreas from NOD (nonobese diabetic) mice, as well as from BALB/c and SJL mice. Expression of MAdCAM-1 was reportedly induced on endothelium in inflamed islets of the pancreas of the NOD mouse, and MAdCAM-1 was the predominant addressin expressed by NOD islet endothelium at early stages of insulitis (Hanninen, A., *et al., J. Clin. Invest., 92:* 2509-2515 (1993)). Further, accumulation of lymphocytes expressing $\alpha4\beta7$ within islets was observed, and MAdCAM-1 was implicated in the binding of lymphoma cells via $\alpha4\beta7$ to vessels from inflamed islets (Hanninen, A., *et al., J. Clin. Invest., 92:* 2509-2515 (1993)).

Examples of inflammatory diseases associated with mucosal tissues which can be treated according to the present method include mastitis (mammary gland), cholecystitis, cholangitis or pericholangitis (bile duct and surrounding tissue of the liver), chronic bronchitis, chronic sinusitis, asthma, and graft versus host disease (e.g., in the gastrointestinal tract). As seen in Crohn's disease, inflammation often extends beyond the mucosal surface, accordingly chronic inflammatory diseases of the lung which result in interstitial fibrosis, such as hypersensitivity pneumonitis, collagen diseases, sarcoidosis, and other idiopathic conditions can be amenable to treatment.

Treatment can be curative, induce remission or quiescence or prevent relapse or recurrence of active disease. According to the method, treatment can be episodic or chronic (e.g., chronic treatment of active disease, to maintain quiescent disease, to induce quiescence and maintain quiescence), for example.

In a particularly preferred embodiment, a human or humanized immunoglobulin having binding specificity for $\alpha4\beta7$ integrin is administered to a human having inflammatory bowel disease, such as ulcerative colitis or Crohn's disease. The immunoglobulin can be administered to treat active disease and/or to maintain quiescence (i.e., inhibit relapse or recurrence). In a particular embodiment, the human

or humanized immunoglobulin can be administered to maintain quiescence of inflammatory bowel disease which has been induced by treatment with one or more other agents (e.g., steroids (prednisone, prednisolone, adrenocorticotrophic hormone (ACTH)), cyclosporin A, FK506, antibody having binding specificity for TNFα (infliximab, CDP571), azathioprene, 6-mercaptopurine, 5-aminosalicylic acid (5-ASA) or compounds containing 5-ASA (e.g., sulfsalazine, olsalazine, balsalazide) antibiotics (e.g., metronidazole), interleukins (IL-10, IL-11), nicotine, heparin, thalidomide, lidocane) or surgery (e.g., intestinal resection).

The human immunoglobulin or antigen-binding fragment thereof, or humanized immunoglobulin or antigen-binding fragment thereof is administered in an effective amount. For therapy, an effective amount is an amount sufficient to achieve the desired therapeutic (including prophylactic) effect (such as an amount sufficient to reduce or prevent $\alpha4\beta7$ integrin-mediated binding to a ligand thereof and/or signalling, thereby inhibiting leukocyte adhesion and infiltration and/or associated cellular responses in an amount sufficient to induce remission or prevent relapse or recurrence of disease). The human immunoglobulin or antigen-binding fragment thereof, or humanized immunoglobulin or antigen-binding fragment thereof can be administered in a single dose or in an initial dose followed by one or more subsequent doses as described herein. The amount of immunoglobulin or antigen-binding fragment administered in a particular dose as well as the interval between doses can depend on the characteristics of the individual, such as general health, age, sex, body weight and tolerance to drugs as well as the type and severity of disease. The skilled artisan will be able to determine appropriate dosages depending on these and other factors.

According to the method, the human or humanized immunoglobulin can be administered to an individual (e.g., a human) alone or in conjunction with another agent (i.e., one or more additional agents). A human or humanized immunoglobulin can be administered before, along with or subsequent to administration of the additional agent. In one embodiment, more than one human or humanized immunoglobulin which inhibits the binding of $\alpha4\beta7$ integrin to its ligands is administered. In another

embodiment, an antibody (e.g, human antibody, humanized antibody), such as an anti-MAdCAM-1, anti-VCAM-1, or anti-ICAM-1 antibody, which inhibits the binding of leukocytes to an endothelial ligand is administered in addition to a human or humanized immunoglobulin which binds $\alpha4\beta7$ integrin. In yet another embodiment, an additional pharmacologically active ingredient (e.g., an antiinflammatory compound, such as 5-aminosalicylic acid (5-ASA) or compounds containing 5-ASA (e.g., sulfsalazine, olsalazine, balsalazide), another non-steroidal antiinflammatory compound, or a steroidal antiinflammatory compound (e.g., prednisone, prednisolone, adrenocorticotrophic hormone (ACTH)), immunosuppressive agents (azathioprene, 6-mercaptopurine, cyclosporin A, FK506), immunomodulators (e.g., antibody having binding specificity for TNFα (infliximab, CDP571), thalidomide, interleukins (e.g., recombinant human IL-10, recombinant human IL-11)), antibiotics (e.g., metronidazole), nicotine, heparin, lidocaine) can be administered in conjunction with a humanized immunoglobulin of the present invention.

A variety of routes of administration are possible, including, but not necessarily limited to, parenteral (e.g., intravenous, intraarterial, intramuscular, intrathecal, subcutaneous injection), oral (e.g., dietary), topical, inhalation (e.g., intrabronchial, intranasal or oral inhalation, intranasal drops), or rectal, depending on the disease or condition to be treated. Parenteral administration, particularly intravenous injection and subcutaneous injection, is preferred.

The human immunoglobulin or antigen-binding fragment thereof and/or the humanized immunoglobulin or antigen-binding fragment thereof can be administered to the individual as part of a pharmaceutical or physiological composition for the treatment of a disease associated with leukocyte infiltration of mucosal tissues (e.g., inflammatory bowel disease (e.g., ulcerative colitis, Crohn's disease). Such a composition can comprise an immunoglobulin or antigen-binding fragment having binding specificity for $\alpha4\beta7$ integrin as described herein, and a pharmaceutically or physiologically acceptable carrier. Pharmaceutical or physiological compositions for co-therapy can comprise an immunoglobulin or antigen-binding fragment having binding specificity for $\alpha4\beta7$

integrin and one or more additional therapeutic agents. An immunoglobulin or antigen-binding fragment having binding specificity for α4β7 integrin function and an additional therapeutic agent can be components of separate compositions which can be mixed together prior to administration or administered separately. Formulation will vary according to the route of administration selected (e.g., solution, emulsion, capsule). Suitable carriers can contain inert ingredients which do not interact with the immunoglobulin or antigen-binding fragment and/or additional therapeutic agent. Standard pharmaceutical formulation techniques can be employed, such as those described in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. Suitable carriers for parenteral administration include, for example, sterile water, physiological saline, bacteriostatic saline (saline containing about 0.9% mg/ml benzyl alcohol), phosphate-buffered saline, Hank's solution, Ringer's-lactate and the like. Methods for encapsulating compositions (such as in a coating of hard gelatin or cyclodextran) are known in the art (Baker, *et al.,* "Controlled Release of Biological Active Agents", John Wiley and Sons, 1986). For inhalation, the agent can be solubilized and loaded into a suitable dispenser for administration (e.g., an atomizer, nebulizer or pressurized aerosol dispenser).

The present invention will now be illustrated by the following Examples, which are not intended to be limiting in any way.

EXAMPLES

Introduction

LDP-02 is a humanized IgG1 monoclonal antibody that binds α4β7 integrin, a cell surface glycoprotein present on the surface of most T and B lymphocytes. α4β7 mediates lymphocyte trafficking to gastrointestinal mucosa and gut-associated lymphoid tissue through adhesion interaction with the homing receptor MAdCAM-1. By blocking α4β7-MAdCAM-1 interactions, LDP-02 can inhibit the recruitment of leukocytes from the vasculature to the gastrointestinal mucosa, thus having a beneficial effect on the inflammatory activity in patients afflicted with inflammatory bowel disease (IBD) such

as ulcerative colitis and Crohn's Disease.

This section presents information from the two LDP-02 clinical trials that have been completed. These trials include one completed Phase I study conducted in healthy subjects (Study L297-007) and one completed Phase Ib/IIa trials in patients with ulcerative colitis (UC)(Study L297-006). Table 1 describes each of the studies.

Table 1

Study No. # Sites Country	Study Status	Study Design/ Population	Dosing Regimen, Dose, Route	Number of Subjects Enrolled
L297-007 1 UK	Completed Start: Jan98 End: Apr98	Phase I, randomized, double-blind, placebo-controlled, ascending single dose study. Healthy Male Subjects 18-50 years of age	Day 1 (single dose) 0.15 mg/kg IV 0.15 mg/kg SC 0.5 mg/kg IV 1.5 mg/kg IV 2.5 mg/kg IV	Total= 19 LDP-02= 14 Placebo= 5
L297-006 5 Canada	Completed Start: Sept98 End: Dec99	Phase Ib/IIa, randomized, double-blind, placebo-controlled, single rising dose, multicenter study. Patients with moderately severe ulcerative colitis. Prior steroid use was limited (≤20mg/day). Use of 5-ASAs was allowed.	Day 1 (single dose) 0.15 mg/kg SC 0.15 mg/kg IV 0.5 mg/kg IV 2.0 mg/kg IV placebo IV	Total= 29 LDP-02= 21 Placebo= 8

Example 1: Study L297-007

Study L297-007 entitled, "A Placebo-Controlled, Double-Blind, Rising Dose Study Investigating the Tolerability, Pharmacodynamics and Pharmacokinetics of LDP-02 Given by the Subcutaneous and Intravenous Routes in Healthy Male Volunteers" has been completed and final results are presented in this section.

Study Design

Study L297-007 was a randomized, double-blind, placebo-controlled, ascending single-dose study in healthy male volunteers. Healthy male volunteers 18 to 50 years of age meeting all inclusion/exclusion criteria were enrolled in the study sequentially by

study group and, within each study group, were randomly assigned to receive LDP-02 or placebo (i.e., isotonic sodium citrate buffer). To minimize risk to subjects, safety and tolerability were reviewed at each dose level prior to escalating to the next dose level. The treatment groups and numbers of subjects planned for the study are shown in Table 2.

Table 2 Study L297-007: Study Groups

Group	Route of Administration*	LDP-02 # subjects	LDP-02 Dose	Placebo # subjects
1	IV	3	0.15 mg/kg	1
	SC	3	0.15 mg/kg	1
2	IV	3	0.5 mg/kg	1
3	IV	3	1.5 mg/kg	1
4	IV	3	2.5 mg/kg	1

*SC= subcutaneous administration; IV = intravenous administration

On study Day 1, LDP-02 or placebo was administered either SC into the thigh (Group 1 SC dosing only) or via a 30 minute constant rate IV infusion (Groups 1-4). Safety assessments included recording of adverse events, physical examinations, vital signs, clinical laboratories (i.e., hematology, blood chemistries, and urinalysis), plasma cytokine levels, and 12-lead electrocardiograms (ECGs). In addition, since this was the first clinical trial of LDP-02, continuous cardiac monitoring was carried out pre-dose through 4 hours post-dose. Blood samples were obtained to assess anti-antibody response to LDP-02, cytokine levels, serum LDP-02 concentration (pharmacokinetics), and saturation and binding site occupation of $\alpha4\beta7$ receptors and lymphocyte subsets (pharmacodynamics). Study assessments were conducted at specified times through 36 days post-treatment. Following the results of the Day 36 pharmacokinetic and pharmacodynamic (immunological) analyses, the protocol was amended to allow additional blood draws for subjects who received LDP-02. These blood draws were used to follow LDP-02 serum levels until they became non-quantifiable (i.e., below the limit of quantification [BLQ]) and to ensure that $\alpha4\beta7$ saturation and memory cell

populations had returned to baseline (pre-dose) levels. This amendment was particularly important in the higher dose groups where the characteristics of terminal phase kinetics were not well established by Day 36.

Study Results

Pharmacokinetics

The assay of LDP-02 in serum was performed using a validated cell-based assay. Standards and samples were incubated with a target cell line (HUT-78) which expresses the $\alpha 4\beta 7$ antigen. After washing, a fluorescently labeled polyclonal anti-human IgG1 was added. Fluorescence intensity was measured by flow cytometry and compared with the fluorescence intensity of LDP-02 standards. The effective serum concentration of LDP-02 was then defined by comparison of the sample with a standard curve generated with known concentrations of LDP-02.

Blood samples for determination of LDP-02 serum concentration were collected pre-dose, 1, 1.5, 3, 8, 12 and 24 hours after dosing, and on Days 3, 5, 7, 8, 15, 22, and 36. When it became known that LDP-02 was still detectable at Day 36, blood draws for subjects who received LDP-02 continued until levels had fallen to below the limits of quantitation of the assay. Thirteen of the 14 subjects who received LDP-02 returned for follow-up blood draws up to a maximum of 226 days post-dose.

LDP-02 concentrations over time by individual patient and mean pharmacokinetic parameters by LDP-02 dose group are presented in the Appendix to Study L297-007. Mean LDP-02 serum concentrations over time are plotted out to the last blood draw for all treatment groups in FIG. 6.

Table 3 Study L297-007: Mean Pharmacokinetic Parameters of LDP-02 in Healthy Subjects[1]

Pharmacokinetic Parameter	Dose and Route of Administration of LDP-02 (number of subjects)				
	0.15 mg/kg SC (n=3)	0.15 mg/kg IV (n=3)	0.5 mg/kg IV (n=3)	1.5 mg/kg IV (n=3)	2.5 mg/kg IV (n=2)
C_{max} (µg/mL)	1.112 (0.519)	7.648 (3.201)	15.760 (7.476)	118.813 (14.544)	101.749 (5.117)
t_{max} (days) (median & range)	6.01 (4.01 - 6.01)	0.13 (0.04 - 0.33)	0.5 (0.06 - 0.5)	0.13 (0.06-0.33)	0.05 (0.04-0.06)
$T_{1/2z}$ (days)	4.33 (2.23)	4.39 (1.51)	4.02 (0.71)	14.9 (10.3)	17.1 (8.91)
AUC_t (µg·day/mL)	10.4 (4.40)	19.5 (5.00)	83.6 (18.3)	660 (229)	1651 (229)
λ_z (1/day)	0.1852 (0.0735)	0.1731 (0.0673)	0.1763 (0.0344)	0.0994 (0.1145)	0.0469 (0.0244)
AUC (µg·day/mL)	11.4 (5.80)	20.3 (5.88)	85.1 (18.2)	755 (308)	1747 (95.8)
AUC Extrapolated %	5.9 (7.3)	3.4 (3.2)	1.8 (1.4)	9.5 (16.1)	5.7 (8.0)
CL* (mLday/kg)	15.3 (6.26)	7.75 (1.93)	6.06 (1.32)	2.31 (1.19)	1.43 (0.08)
V_z* (mL/kg)	82.5 (6.88)	46.6 (10.1)	34.3 (2.84)	54.0 (51.4)	35.9 (20.3)

[1]All values are mean +/- SD unless otherwise indicated. The SD appears in parenthesis. *Clearance and volume terms for the SC dose group are the apparent clearance (CL/F) and apparent volume (V_z/F).

Values were obtained for the mean single dose IV pharmacokinetic parameters for the 4 dose groups (C_{max}, $t_{1/2z}$ and AUC). Follow-up samples (i.e., those taken beyond Day 36), where the focus was on safety, allowed some further characterization of the concentration-time profiles. The difference in the $t_{1/2z}$ values between the 2 lower dose groups (0.15 and 0.5 mg/kg) and the higher dose groups (1.5 and 2.5 mg/kg) of around 10 days could be explained in that the "true" terminal phase for the higher dose groups had not been characterized. The non-compartmental pharmacokinetics of the lower doses of LDP-02 (0.15 and 0.5 mg/kg) were well characterized and non-linear pharmacokinetics became evident as the dose was increased up to 2.5 mg/kg.

Assessment of the Pharmacodynamic Effect of LDP-02

Fluorescent activated cell scanning (FACS) analysis was used to measure the

presence of $\alpha4\beta7$ sites on peripheral blood lymphocytes pre- and post-LDP-02 administration. To detect $\alpha4\beta7$ that were recognized by antibody, biotin labeled ACT-1, the murine homologue of LDP-02, was added to samples of patient blood and detected using PE-streptavidin. The standardized mean equivalent soluble fluorescence (MESF) is proportional to the number of detectable $\alpha4\beta7$ sites.

Serum $\alpha4\beta7$ binding over time (MESF values and percentage of baseline at each post-dose time point) are presented by individual subject and by treatment group in the Appendix to Study L297-007.

As measured by FACS analysis, mean saturation of $\alpha4\beta7$ integrin on lymphocytes over time (i.e., to Day 36) for each treatment are presented in FIG. 7.

As seen in FIG. 7, there was no detection of free $\alpha4\beta7$ binding sites on lymphocytes for at least two weeks following administration of all LDP-02 doses. Between about day 7 and day 22, $\alpha4\beta7$ signal started to return to baseline for the 0.15 mg/kg IV dose group and for the 0.15 mg/kg SC dose group. Between day 22 and day 36, a4b7 signal started to return to baseline for the 0.5 mg/kg IV dose group. At the higher doses of LDP-02 studied (1.5, and 2.5 mg/kg) loss of $\alpha4\beta7$ signal persisted for longer than 36 days following single IV doses. For the 2.5 mg/kg dose group, $\alpha4\beta7$ binding saturation continued up to Day 70 (see, data in Appendix to Study L297-007).

Follow-up blood sampling up to about Study Day 200 was done to confirm that free $\alpha4\beta7$ binding sites on lymphocytes has returned to baseline (pre-dose) levels. The initial reappearance of free $\alpha4\beta7$ sites appeared to occur when LDP-02 blood concentrations became non-detectable.

Conclusions

The administration of LDP-02 at IV doses of 0.15, 0.50, 1.50, and 2.5 mg/kg and a SC dose of 0.15 mg/kg to healthy male subjects was well-tolerated.

Following administration of all LDP-02 doses there was no detection of free $\alpha4\beta7$ binding sites on lymphocytes for approximately two weeks post-dose. Saturation of $\alpha4\beta7$ binding sites continued for up to approximately 2 weeks post-dosing for the

0.15 mg/kg IV group and for up to approximately 3 weeks post-dosing for the 0.15 mg/kg SC and 0.5 mg/kg IV groups. Duration of effect persisted for a month or longer with the 1.5 mg/kg IV dose and continued to approximately Day 70 with 2.5 mg/kg LDP-02 IV. Follow-up samples obtained after Day 36 demonstrated that expression of free $\alpha4\beta7$ binding sites had returned to baseline (pre-dose levels). No anti-idiotype antibodies were raised to LDP-02 indicating that it did not initiate a humoral immunogenic response. The non-compartmental pharmacokinetics of the lower doses of LDP-02 (0.15 and 0.5 mg/kg) became evident as the dose was increased up to 2.5 mg/kg.

APPENDEX TO STUDY L297-007

LDP-02 Serum Concentration Over Time by Subject by Treatment Group. Data from individual patients are presented in Tables 4-9.

Table 4 0.15 mg/kg LDP-02 IV

Subject #2			Subject # 3			Subject # 4			Mean
Time (hr)	Time (day)	μg/mL	Time (hr)	Time (day)	μg/mL	Time (hr)	Time (day)	μg/mL	μg/mL (n=3)
Pre-Dose	Pre-Dose	0.01	Pre-Dose	Pre-Dose	0.01	Pre-Dose	Pre-Dose	0.01	0.01
1.0	0.042	5.24	1.0	0.042	7.98	1.0	0.042	2.48	5.24
1.5	0.063	5.33	1.5	0.063	6.21	1.5	0.063	3.42	4.99
3.0	0.125	5.47	3.0	0.125	4.66	3.0	0.125	4.29	4.81
8.0	0.333	10.67	8.0	0.333	5.10	8.0	0.333	3.26	6.34
12.0	0.500	4.49	12.0	0.500	4.50	12.0	0.500	2.42	3.80
24.0	1.000	3.23	24.0	1.000	3.63	24.0	1.000	2.24	3.03
72.0	3.000	1.84	72.0	3.000	2.94	72.0	3.000	3.05	2.61
120.0	5.000	1.21	120.0	5.000	1.84	120.0	5.000	1.16	1.40
168.0	7.000	0.94	168.0	7.000	1.29	168.0	7.000	0.74	0.99
192.0	8.000	0.62	192.0	8.000	1.13	192.0	8.000	0.70	0.82
360.0	15.000	0.04	360.0	15.000	0.53	360.0	15.000	0.26	0.28
528.0	22.000	0.02	528.0	22.000	0.21	528.0	22.000	0.09	0.10
864.0	36.000	0.02	864.0	36.000	0.01	864.0	36.000	0.01	0.01
			3912.0	163.000	0.01	3912.0	163.000	0.01	0.01
			4920.0	205.000	0.01	4752.0	198.000	0.01	0.01

Table 5 0.15 mg/kg LDP-02 SC

Subject # 5			Subject # 6			Subject # 8			Mean
Time (hr)	Time (day)	μg/mL	Time (hr)	Time (day)	μg/mL	Time (hr)	Time (day)	μg/mL	μg/mL (n=3)
Pre-Dose	Pre-Dose	0.01	Pre-Dose	Pre-Dose	0.01	Pre-Dose	Pre-Dose	0.01	0.01
1.0	0.042	0.01	1.0	0.042	0.01	1.0	0.042	0.01	0.01
1.5	0.063	0.01	1.5	0.063	0.01	1.5	0.063	0.01	0.01
3.0	0.125	0.01	3.0	0.125	0.01	3.0	0.125	0.01	0.01
8.0	0.333	0.06	8.0	0.333	0.09	8.0	0.333	0.09	0.08
12.0	0.500	0.11	12.0	0.500	0.12	12.0	0.500	0.10	0.11
24.0	1.000	0.12	24.0	1.000	0.30	24.0	1.000	0.55	0.32
72.0	3.000	0.23	72.0	3.000	0.81	72.0	3.000	0.91	0.65
120.0	5.000	0.54	120.0	5.000	0.93	120.0	5.000	1.13	0.86
168.0	7.000	0.71	168.0	7.000	0.88	168.0	7.000	1.70	1.10
192.0	8.000	0.62	192.0	8.000	0.81	192.0	8.000	1.05	0.83
360.0	15.000	0.28	360.0	15.000	0.08	360.0	15.000	0.53	0.30
528.0	22.000	0.02	528.0	22.000	0.03	528.0	22.000	0.26	0.11
864.0	36.000	0.04	864.0	36.000	0.04	864.0	36.000	0.01	0.03
3912.0	163.000	0.01	3912.0	163.000	0.01	3912.0	163.000	0.01	0.01
5088.0	212.000	0.01	5088.0	212.000	0.01	5088.0	212.000	0.01	0.01

Table 6 0.5 mg/kg LDP-02 IV

Subject # 9			Subject # 10			Subject # 12			Mean
Time (hr)	Time (day)	μg/mL	Time (hr)	Time (day)	μg/mL	Time (hr)	Time (day)	μg/mL	μg/mL (n=3)
Pre-Dose	Pre-Dose	0.01	Pre-Dose	Pre-Dose	0.01	Pre-Dose	Pre-Dose	0.01	0.01
1.0	0.042	9.06	1.0	0.042	10.74	1.0	0.042	10.93	10.24
1.5	0.063	24.39	1.5	0.063	6.62	1.5	0.063	8.17	13.06
3.0	0.125	16.37	3.0	0.125	10.14	3.0	0.125	9.94	12.15
8.0	0.333	15.04	8.0	0.333	9.30	8.0	0.333	9.35	11.23
12.0	0.500	10.64	12.0	0.500	11.70	12.0	0.500	11.19	11.18
24.0	1.000	9.17	24.0	1.000	9.00	24.0	1.000	8.52	8.90
72.0	3.000	5.34	72.0	3.000	7.55	72.0	3.000	7.60	6.83
120.0	5.000	10.25	120.0	5.000	2.43	120.0	5.000	8.58	7.09
168.0	7.000	5.74	168.0	7.000	6.59	168.0	7.000	4.93	5.75
192.0	8.000	3.79	192.0	8.000	2.48	192.0	8.000	4.32	3.53
360.0	15.000	1.70	360.0	15.000	2.21	360.0	15.000	2.49	2.13
528.0	22.000	0.41	528.0	22.000	0.12	528.0	22.000	1.65	0.73
864.0	36.000	0.01	864.0	36.000	0.01	864.0	36.000	0.11	0.04
3576.0	149.00	0.01	3912.0	163.000	0.01	3576.0	149.000	0.01	0.01
			5424.0	226.000	0.01				0.01

Table 7 1.5 mg/kg LDP-02 IV

Subject # 13			Subject # 15			Subject # 16			Mean
Time (hr)	Time (day)	µg/mL	Time (hr)	Time (day)	µg/mL	Time (hr)	Time (day)	µg/mL	µg/mL (n=3)
Pre-Dose	Pre-Dose	0.01	Pre-Dose	Pre-Dose	0.01	Pre-Dose	Pre-Dose	0.01	0.01
1.0	0.042	87.62	1.0	0.042	58.06	1.0	0.042	103.10	82.93
1.5	0.063	63.67	1.5	0.063	134.97	1.5	0.063	86.05	94.90
3.0	0.125	92.78	3.0	0.125	63.78	3.0	0.125	106.78	87.78
8.0	0.333	114.69	8.0	0.333	64.12	8.0	0.333	84.42	87.74
12.0	0.500	73.02	12.0	0.500	43.76	12.0	0.500	44.09	53.62
24.0	1.000	99.61	24.0	1.000	77.77	24.0	1.000	71.80	83.06
72.0	3.000	102.88	72.0	3.000	38.82	72.0	3.000	67.61	69.77
120.0	5.000	42.46	120.0	5.000	25.26	120.0	5.000	23.95	30.56
168.0	7.000	26.10	168.0	7.000	18.42	168.0	7.000	23.85	22.79
192.0	8.000	46.47	192.0	8.000	11.90	192.0	8.000	19.85	26.07
360.0	15.000	19.83	360.0	15.000	5.80	360.0	15.000	19.54	15.06
528.0	22.000	10.93	528.0	22.000	0.11	528.0	22.000	13.89	8.31
864.0	36.000	0.19	864.0	36.000	0.69	864.0	36.000	9.49	3.46
1968.0	82.000	0.48	1968.0	163.000	0.30				0.39
3264.0	136.000	0.01	3264.0	212.000	0.03				0.02
4272.0	178.000	0.01				3960.0	165.000	0.01	0.01
			4824.0	201.000	0.01				0.01

Table 8 2.5 mg/kg LDP-02 IV

Subject # 18			Subject # 19			Mean
Time (hr)	Time (day)	µg/mL	Time (hr)	Time (day)	µg/mL	µg/mL (n=2)
Pre-Dose	Pre-Dose	0.01	Pre-Dose	Pre-Dose	0.01	0.01
1.0	0.042	105.37	1.0	0.042	84.06	94.72
1.5	0.063	71.27	1.5	0.063	98.13	84.70
3.0	0.125	73.49	3.0	0.125	81.59	77.54
8.0	0.333	84.00	8.0	0.333	80.17	82.09
12.0	0.500	103.81	12.0	0.500	85.53	94.67
24.0	1.000	68.79	24.0	1.000	85.52	77.15
72.0	3.000	63.30	72.0	3.000	69.49	66.40
120.0	5.000	53.33	120.0	5.000	59.11	56.22
168.0	7.000	50.72	168.0	7.000	54.63	52.67
192.0	8.000	43.47	192.0	8.000	67.32	55.40
360.0	15.000	22.82	360.0	15.000	23.85	23.34
528.0	22.000	22.45	528.0	22.000	21.92	22.19
864.0	36.000	17.42	864.0	36.000	20.63	19.03
1680.0	70.000	5.48	1656.0	69.000	4.63	5.06
3312.0	138.000	0.01	2976.0	124.000	0.08	0.04
3984.0	166.000	0.01	3648.0	152.000	0.01	0.01
			4536.0	189.000	0.01	0.01

Table 9 placebo group

Time (hr)	Time (day)	Subject # 1	Subject # 7	Subject # 11	Subject # 14	Subject # 17
Pre-Dose	Pre-Dose	Its	Its	Its	Its	Its
1.0	0.042	Its	Its	Its	Its	Its
1.5	0.063	Its	Its	Its	Its	Its
3.0	0.125	Its	Its	Its	Its	Its
8.0	0.333	Its	Its	Its	Its	Its
12.0	0.500	Its	Its	Its	Its	Its
24.0	1.000	Its	Its	Its	Its	Its
72.0	3.000	Its	Its	Its	Its	Its
120.0	5.000	Its	Its	Its	Its	Its
168.0	7.000	Its	Its	Its	Its	Its
192.0	8.000	Its	Its	Its	Its	Its
360.0	15.000	Its	Its	Its	Its	Its
528.0	22.000	Its	Its	Its	Its	Its
864.0	36.000	Its	Its	Its	Its	Its

Its = below the limit of detection

Study L297-007: Mean Pharmacokinetic Parameters by Treatment Group Data from individual patients are presented in Tables 10-14.

Table 10 0.15 mg/kg LDP-02 IV

Subject	C_{max} (μg/ml)	t_{max} (days)	AUC_t (μg·day/ml)	λ_z (1/day)	$t_{½z}$ (days)	AUC (μg·day/ml)	AUC_{ext} (%)	V_z (ml/kg)	CL (ml/day/kg)
2	10.667	0.33	16.4	0.2486	2.79	16.5	0.3	36.7	9.11
3	7.984	0.04	25.3	0.1196	5.79	27.1	6.7	46.3	5.53
4	4.292	0.13	16.9	0.1510	4.59	17.5	3.3	56.9	8.60
Mean	7.648	0.13*	19.5	0.1731	4.39	20.3	3.4	46.6	7.75
SD	3.201		5.00	0.0673	1.51	5.88	3.2	10.1	1.93

*Median value

C_{max} = maximum concentration

t_{max} = time to maximum concentration

λ_z = a measure of elimination

$t_{1/2z}$ = terminal half-live

AUC_t = AUC_{all} = area under the curve using all time points

AUC = AUC_{ext} = area under curve extrapolated

AUC ext (%) = % of area under curve attributed to extrapolation extrapolation

V_z = apparent volume of distribution

CL = Clearance

Table 11 0.15 mg/kg LDP-02 SC

Subject	C_{max} (μg/ml)	t_{max} (days)	AUC_t (μg·day/ml)	λ_z (1/day)	$t_{½z}$ (days)	AUC (μg·day/ml)	AUC_{ext} (%)	V_z (ml/kg)	CL (ml/day/kg)
5	0.711	6.01	7.18	0.2298	3.02	7.32	2.0	89.1	20.5
6	0.927	4.01	8.71	0.2253	3.08	8.83	1.4	75.4	17.0
8	1.699	6.01	15.4	0.1003	6.91	18.0	14.3	82.9	8.32
Mean	1.112	6.01*	10.4	0.1852	4.33	11.4	5.9	82.5	15.3
SD	0.519		4.40	0.0735	2.23	5.80	7.3	6.88	6.26

*Median value

Table 12 0.5 mg/kg LDP-02 IV

Subject	C_{max} (μg/ml)	t_{max} (days)	AUC_t (μg·day/ml)	λ_z (1/day)	$t_{½z}$ (days)	AUC (μg·day/ml)	AUC_{ext} (%)	V_z (ml/kg)	CL (ml/day/kg)
9	24.388	0.06	82.2	0.1586	4.37	85.1	3.4	37.0	5.87
10	11.699	0.50	66.1	0.2159	3.21	67.0	1.3	34.6	7.47
12	11.194	0.50	102.5	0.1543	4.49	103	0.8	31.4	4.84
Mean	15.760	0.50*	83.6	0.1763	4.02	85.1	1.8	34.3	6.06
SD	7.476		18.3	0.0344	0.71	18.2	1.4	2.84	1.32

*Median value

Table 13 1.5 mg/kg LDP-02 IV

Subject	C_{max} (μg/ml)	t_{max} (days)	AUC_t (μg·day/ml)	λ_z (1/day)	$t_{½z}$ (days)	AUC (μg·day/ml)	AUC_{ext} (%)	V_z (ml/kg)	CL (ml/day/kg)
13	114.686	0.33	854	0.2316	2.99	855	0.1	7.58	1.75
15	134.975	0.06	408	0.0336	20.6	409	0.2	109	3.67
16	106.779	0.13	719	0.0331	20.9	1000	28.1	45.3	1.50
Mean	118.813	0.13*	660	0.0994	14.9	755	9.5	54.0	2.31
SD	14.544		229	0.1145	10.3	308	16.1	51.4	1.19

*Median value

Table 14 2.5 mg/kg LDP-02 IV

Subject	C_{max} (μg/ml)	t_{max} (days)	AUC_t (μg·day/ml)	λ_z (1/day)	$t_{½z}$ (days)	AUC (μg·day/ml)	AUC_{ext} (%)	V_z (ml/kg)	CL (ml/day/kg)
18	105.367	0.04	1489	0.0296	23.4	1680	11.3	50.2	1.49
19	98.131	0.06	1814	0.0642	10.8	1815	0.1	21.5	1.38
Mean	101.749	0.05*	1651	0.0469	17.1	1747	5.7	35.9	1.43
SD	5.117		229	0.0244	8.91	95.8	8.0	20.3	0.08

*Median value

L297-007: Serum α4β7 Binding Over Time by Subject by Treatment Group. Data from individual patients are presented in Tables 15-20. For each subject the time of blood sampling, MESF of the sample and % of baseline (pre-dose) MESF is presented.

Table 15 0.15 mg/kg LDP-02 IV

Subject # 2			Subject # 3			Subject # 4			Mean	
Pre-Dose	5689	100%	Pre-Dose	5424	100%	Pre-Dose	4177	100%	5097	100%
3 hr	605	11%	3 hr	591	11%	3 hr	588	14%	595	12%
24 hrs	589	10%	24 hrs	600	11%	24 hrs	631	15%	607	12%
Day 3	501	9%	Day 3	496	9%	Day 3	548	13%	515	10%
Day 7	474	8%	Day 7	473	9%	Day 7	512	12%	487	10%
Day 15	1819	32%	Day 15	578	11%	Day 15	599	14%	999	20%
Day 22	2426	43%	Day 22	558	10%	Day 22	609	15%	1198	23%
Day 36	3028	53%	Day 36	3570	66%	Day 36	3469	83%	3356	66%
			Day 163	6934	128%	Day 163	6837	164%	6885	135%
			Day 205	4675	86%	Day 205	6755	162%	5715	112%

Table 16 0.15 mg/kg LDP-02 SC

Subject # 5			Subject # 6			Subject # 8			Mean	
Pre-Dose	6043	100%	Pre-Dose	6779	100%	Pre-Dose	5857	100%	6226	100%
3 hr	1797	30%	3 hr	4727	70%	3 hr	1514	26%	2679	43%
24 hrs	637	11%	24 hrs	588	9%	24 hrs	616	11%	614	10%
Day 3	529	9%	Day 3	520	8%	Day 3	527	9%	525	8%
Day 7	486	8%	Day 7	474	7%	Day 7	485	8%	482	8%
Day 15	598	10%	Day 15	642	9%	Day 15	635	11%	625	10%
Day 22	759	13%	Day 22	934	14%	Day 22	579	10%	757	12%
Day 36	1455	24%	Day 36	1452	21%	Day 36	2799	48%	1902	31%
Day 163	2743	45%	Day 163	1989	29%	Day 163	4621	79%	3118	50%
Day 212	4201	70%	Day 212	2601	38%	Day 212	4832	82%	3878	62%

Table 17 0.5 mg/kg LDP-02 IV

Subject # 9			Subject # 10			Subject # 12			Mean	
Pre-Dose	5519	100%	Pre-Dose	5966	100%	Pre-Dose	8550	100%	6678	100%
3 hr	533	10%	3 hr	548	9%	3 hr	539	6%	540	8%
24 hrs	542	10%	24 hrs	554	9%	24 hrs	527	6%	541	8%
Day 3	565	10%	Day 3	574	10%	Day 3	539	6%	560	8%
Day 7	544	10%	Day 7	551	9%	Day 7	547	6%	547	8%
Day 15	540	10%	Day 15	525	9%	Day 15	520	6%	528	8%
Day 22	555	10%	Day 22	572	10%	Day 22	543	6%	557	8%
Day 36	885	16%	Day 36	1182	20%	Day 36	643	8%	903	14%
Day 149	4448	81%	Day 163	5256	88%	Day 149	7810	91%	5838	87%

Table 18 1.5 mg/kg LDP-02 IV

Subject # 13			Subject # 15			Subject # 16			Mean	
Pre-Dose	4966	100%	Pre-Dose	5544	100%	Pre-Dose	5622	100%	5378	100%
3 hr	518	10%	3 hr	539	10%	3 hr	545	10%	534	10%
24 hrs	482	10%	24 hrs	487	9%	24 hrs	520	9%	496	9%
Day 3	511	10%	Day 3	475	9%	Day 3	514	9%	500	9%
Day 7	549	11%	Day 7	535	10%	Day 7	569	10%	551	10%
Day 15	472	9%	Day 15	474	9%	Day 15	491	9%	479	9%
Day 22	603	12%	Day 22	617	11%	Day 22	576	10%	599	11%
Day 36	618	12%	Day 36	866	16%	Day 36	606	11%	697	13%
Day 82	922	19%	Day 80	832	15%				877	16%
Day 134	1647	33%	Day 134	1531	28%				1589	30%
Day 176	2322	47%							2322	43%

Table 19 2.5 mg/kg LDP-02 IV

Subject # 18			Subject # 19			Mean	
Pre-Dose	5922	100%	Pre-Dose	5065	100%	5494	100%
3 hr	527	9%	3 hr	527	10%	527	10%
24 hrs	568	10%	24 hrs	571	11%	569	10%
Day 3	511	9%	Day 3	521	10%	516	9%
Day 7	503	9%	Day 7	513	10%	508	9%
Day 15	530	9%	Day 15	544	11%	537	10%
Day 22	588	10%	Day 22	595	12%	591	11%
Day 36	550	9%	Day 36	554	11%	552	10%
Day 70	615	10%	Day 69	566	11%	590	11%
Day 138	4572	77%	Day 124	1103	22%	2837	52%
Day 166	5603	95%	Day 152	4094	81%	4849	88%

Table 20 placebo group

	Subject # 1		Subject # 7		Subject # 11		Subject # 14		Subject # 17	
Pre-Dose	5807	100%	5198	100%	8747	100%	7017	100%	5982	100%
3 hr	5630	97%	4305	83%	8454	97%	6208	88%	5520	92%
24 hrs	6672	115%	4347	84%	8033	92%	6699	95%	5410	90%
Day 3	6078	105%	4008	77%	8701	99%	6141	88%	5488	92%
Day 7	5617	97%	4047	78%	8668	99%	6327	90%	5194	87%
Day 15	5797	100%	4758	92%	7516	86%	4851	69%	5759	96%
Day 22	5164	89%	4318	83%	6924	79%	5246	75%	5922	99%
Day 36	6200	107%	4686	90%	7065	81%	7857	112%	5349	89%

Example 2. Study L297-006

The study entitled, "A Single Dose Phase Ib/IIa, Placebo Controlled, Randomized, Double-Blind Study to Determine the Safety, Tolerability, Pharmacokinetics, Pharmacodynamics, and Effectiveness of LDP-02 in Patients with Moderately Severe Ulcerative Colitis" was completed and final certain results are presented in this section.

Study Rationale

Results from the Phase I trial (Example 1. Study L297-007) in healthy volunteers showed LDP-02 at doses of 0.15 mg/kg SC and IV, 0.5 mg/kg IV, 1.5 mg/kg IV, and 2.5 mg/kg IV was safe and well-tolerated. In addition, doses of 0.15 mg/kg IV or SC and 0.5 mg/kg IV were shown to have a $t_{½}$ of approximately 100 to 130 hours and flow cytometry data showed that unbound $\alpha4\beta7$ begins to reappear in the 0.15 mg/kg dosage groups approximately two weeks after dosing. Based upon these data, LDP-02 dosages of 0.15 mg/kg SC, 0.15 mg IV, 0.5 mg/kg IV, and 2.0 mg/kg IV were selected for use in the initial study in patients with ulcerative colitis. This study was designed so that each dose of LDP-02 was determined to be safe and well-tolerated prior to escalation to the next dose level.

Study Design

The study was a randomized, double-blind, placebo-controlled, ascending single-dose study in patients diagnosed with moderately-severe ulcerative colitis. Patients with a documented diagnosis of ulcerative colitis with a minimum disease extent of 25 cm from the anal verge were potentially eligible for the study. Patients with severe ulcerative colitis as defined by Truelove-Witts criteria (*Br Med J; 2:1042-1048* (1955)) were excluded. Ulcerative colitis patients who met all inclusion/exclusion criteria were enrolled sequentially into four study groups and, within each study group, were randomly assigned to receive LDP-02 or placebo (i.e., 0.9% sodium chloride). Treatment groups and numbers of patients enrolled are shown

in Table 21.

Table 21: Study Groups

Group	Route of Administration*	LDP-02		Placebo
		# patients	Dose	# patients
1	SC	5	0.15 mg/kg	2
2	IV	5	0.15 mg/kg	2
3	IV	5	0.5 mg/kg	2
4	IV	5	2.0 mg/kg	2

Study medication (LDP-02 or placebo) was administered on Day 1 either SC into the thigh or via a 30 minute IV infusion. Safety assessments included recording of adverse events, physical examinations, vital signs, clinical laboratories (i.e., hematology, blood chemistries, and urinalysis), plasma cytokine levels, and ECGs. Blood was drawn at various time points to measure LDP-02 serum concentrations and to assess the effectiveness of LDP-02 to saturate and block $\alpha 4\beta 7$ binding receptors on peripheral blood lymphocytes. The effectiveness of LDP-02 to reduce inflammation in the colon was measured by clinical disease observations, endoscopic appearance, histopathology, and immunohistochemistry.

Study Results

Patient Disposition and Demography

Twenty-nine patients with moderately-severe ulcerative colitis were entered into the study and 28 completed the trial. One patient was found to be *Clostridium difficile* toxin positive at screening, but due to a delay in reporting laboratory results the patient was admitted into the trial and received 2.0 mg/kg IV of

LDP-02. Once the laboratory results were obtained, the patient was treated with antibiotics and replaced by another patient. There were no other patients discontinued from the study. As patients were recruited into the study over time, there was no attempt to balance the treatment groups with regard to baseline ulcerative colitis history. As such, severity and duration of ulcerative colitis disease and prior medications for ulcerative colitis varied from patient to patient and from treatment group to treatment group. These data are presented in Table 22.

Table 22: Ulcerative Colitis History by Treatment Group

Treatment Group	Time Since Onset of UC Symptoms (yrs)[1]	Time Since Diagnosis of UC (yrs)[1]	# of Acute Exacerbations in past 12 months[1]	Weeks on continuous oral 5-ASA in past 6 months[1]	Weeks on continuous oral steroids in past 6 months[1]
0.15 mg/kg SC (n=5)	5.32 (4.8,6.4)	4.6 (4.3,6.4)	3 (1,12)	24.0 (3,26)	0 (0,6)
0.15 mg/kg IV (n=5)	9.58 (2.6,14.2)	4.9 (2.1,14.0)	1 (1,3)	24.0 (6,26)	10 (0,24)
0.5 mg/kg IV (n=5)	10.8 (0.4,11.8)	9.0 (0.3,11.8)	1 (1,2)	26.0 (0,26)	0 (0,15)
2.0 mg/kg IV (n=6)	9.34 (3.4,58.8)	7.65 (3.2,19.4)	2 (1,5)	25.0 (0,26)	5 (0,26)
All LDP-02 (n=21)	5.99 (0.4,58.8)	4.9 (0.3,19.4)	2 (1,12)	26.0 (0,26)	0 (0,26)
Placebo (n=8)	5.27 (0.4,11.0)	4.85 (0.3,9.7)	1.5 (1,4)	24.0 (0,26)	16 (0,26)

[1]Median values

Disease Measurements

Although this was primarily a dose-ranging safety and pharmacokinetics study, various parameters were measured to assess effectiveness of treatment. Effectiveness assessments included recording changes from baseline using a modified Baron's (endoscopy) Scoring System, the Mayo Clinic Disease Activity Index Score, the Powell-Tuck Disease Activity Index Score, stool frequency, and the Inflammatory

Bowel Disease Questionnaire. Changes from baseline to Day 30 for these parameters are shown in Table 23. For patients in which there was no Day 30 evaluation, the last post-baseline observation obtained was carried forward to Day 30.

Table 23: Change from Baseline to Day 30 in Disease Parameters

Treatment Group	Change from baseline to Day 30[1]				
	Endoscopic Severity Score	Mayo Clinic Activity Index	Powell-Tuck Activity Index	Stool Frequency	Total IBDQ
0.15 mg/kg SC (n=5)	0 (-2,0)	-3.0 (-9,0)	-3.0 (-6,-2)	-1.0 (-7,1)	14.0 (14,72)
0.15 mg/kg IV (n=5)	0 (0,1)	-1.0 (-3,2)	0 (-3,3)	-0.4 (-5,2)	8.0 (-3,95)
0.5 mg/kg IV (n=5)	-2.0 (-3,0)	-10 (-11,0)	-6.0 (-13,-2)	-5.3 (-6,0)	37.0 (14,80)
2.0 mg/kg IV (n=6)	-0.5 (-2,1)	-2.0 (-6,3)	-1.5 (-5,-5)	-3.2 (-8,2)	-2.5 (-59,95)
All LDP-02 (n=21)	0 (-3,1)	-3.0 (-11,3)	-3.0 (-13,5)	-2.4 (-8,2)	14.0 (-59,95)
Placebo (n=8)	-1.0 (-3,2)	-5.0 (-8,4)	-6.0 (-9,-4)	-3.2 (-12,2)	53.5 (-30,82)

[1]Median values and range. For patients without a Day 30 evaluation the last post-baseline evaluation was carried forward to Day 30.

As seen from the results presented in Table 23, there was variability in response among the different treatment groups. The patients receiving 0.5 mg/kg IV appeared to have the best responses; the median endoscopic severity score was reduced

by two grades and the Mayo Clinic score was reduced by 10 points with a decrease in stool frequency. Three of the five patients receiving 0.5 mg/kg IV had a two point improvement in the modified Baron sigmoidoscopy score which is considered an endoscopic response; only one patient (compared with a total of five treated per group) in both the 2.0 mg/kg IV and 0.15 mg/kg SC groups had an endoscopic response. The placebo group also experienced an improvement in sigmoidoscopic score and Mayo Clinic score, although both were less in magnitude when compared to the 0.5 mg/kg IV group. Two of the eight patients experienced an endoscopic response.

The number of patients with a complete remission, defined as a zero on the modified Baron sigmoidoscopic score and on the Mayo Clinic score at Day 30, are reported in Table 24.

Table 24: Patients in Complete Remission at Day 30

Treatment Group	Measured at Day 30[1]	
	Number of Complete Patients	Percentage in Complete Remission
0.15 mg/kg SC (n=5)	0	0
0.15 mg/kg IV (n=5)	0	0
0.5 mg/kg IV (n=5)	2	40%
2.0 mg/kg IV (n=6)	0	0
All LDP-02 (n=21)	2	9.5%
Placebo (n=8)	0	0

[1] Zero on the modified Baron Score and the Mayo Clinic Score in Day 30 results

None of the patients in the placebo group experienced a complete remission while two patients among those receiving LDP-02 had complete remissions. The two patients both were in the same group; both patients received a single administration of 0.5 mg/kg of LDP-02. One of the patients was receiving concurrent mesalamine therapy, while the other was receiving concurrent low dose corticosteroid (20 mg prednisone per day orally).

Pharmacokinetics

The assay of LDP-02 in serum was performed by Cytometry Associates, Inc. as previously described (Study L297-007). Blood samples were collected prior to and immediately following the completion of infusion (Day 1) and on Days 2, 3, 5, 10, 14, 21, 30 and 60 to assess the pharmacokinetic profile of LDP-02.

LDP-02 concentrations over time by individual patient and mean pharmacokinetic parameters by LDP-02 dose are presented in the Appendix to study L296-006.

As seen in FIG. 8, serum levels of LDP-02 for the 0.15 mg/kg IV and SC groups fall to <1.0 μg/mL to approximately 20 days post-dose. For the 2.0 mg/kg dose group, LDP-02 levels remain elevated out to approximately Day 60. Table 25 presents the key pharmacokinetic parameters by treatment group.

Table 25: Pharmacokinetic Parameters of LDP-02

Pharmacokinetic Parameter[1]	Dose and Route of Administration of LDP-02 (number of subjects with data)[2]			
	0.15 mg/kg SC (n=5)	0. 15 mg/kg IV (n=5)	0. 5 mg/kg IV (n=5)	2. 0 mg/kg IV (n=4)[3]
C_{max} (µg/mL)	1.44 (0.33)	3.602 (0.958)	10.544 (2.582)	32.933 (3.360)
t_{max} (days) (median & range)	5 (3-10)	0.13 (0.13-0.13)	0.13 (0.13-0.13)	0.13 (0.13-2)
$t_{½z}$ (days)	15.63 (15.92)	18.91 (20.97)	10.62 (5.23)	15.0 (5.36)
AUC_{all} (µg·day/mL)	25 (16)	27 (11)	91 (32)	515 (93)
λ_z (1/day)	0.1226 (0.1064)	0.0879 (0.0757)	0.0927 (0.0775)	0.0542 (0.0298)
AUC(INF) (µg·day/mL)	31 (23)	34 (18)	100 (39)	553 (116)
CL[4] (mLday/kg)	9.21 (9.54)	7.75 (1.93)	6.06 (1.32)	2.31 (1.19)
V_z[4] (mL/kg)	95.08 (54.19)	101.05 (62.87)	77.63 (30.90)	76.64 (20.03)

[1]All values are mean +/- SD unless otherwise indicated. The SD appears in parenthesis.

[2]Two patients, one in the 0.15 mg/kg SC and one in the 0.5 mg/kg IV groups had evaluable data through Study Day 21 with measurement at later times which were not physiologically possible.

[3]One patient in the 2.0 mg/kg IV dosing group was withdrawn at Study Day 10 and had a surgical intervention. The pharmacokinetic results for this patient are not included.

[4]Clearance and volume terms for the SC dose group are the apparent clearance (CL/F) and apparent volume (V_z/F).

There does appear to be linearity with dose for the maximum concentration of LDP-02 and the area under the curve measured after IV administration. The clearance and the terminal elimination half life appear to be independent of IV dose administered. The volume of distribution appears to decrease slightly with increasing doses of IV LDP-02.

Assessment of the Pharmacodynamic Effect of LDP-02

FACS analysis to measure the presence of $\alpha4\beta7$ sites on blood lymphocytes was previously described (Study L296-007). Serum $\alpha4\beta7$ binding over time (i.e., MESF values and percentage of baseline at each post-dose time point) are presented by individual patient and by treatment group in the Appendix to Study L297-006.

Mean percent of baseline MESF over time for all treatments are presented in FIG. 9. As seen in FIG. 9, percent of baseline MESF rapidly falls to approximately 10% after SC and IV administration of LDP-02 with duration of effect dependent upon dose. Starting at about day 10, $\alpha4\beta7$ signal started to return to baseline for the 0.15 mg/kg IV and SC dose groups. However, $\alpha4\beta7$ signal started to return to baseline between day 30 and day 60 for the 0.5 mg/kg IV and 2.0 mg/kg dose groups.

Conclusions

Administration of LDP-02 at doses of 0.15 mg/kg IV and SC, 0.5 mg/kg IV, and 2.0 mg/kg IV to patients with moderately-severe ulcerative colitis was well-tolerated.

The pharmacokinetic and pharmacodynamic data from patients with ulcerative colitis showed results were consistent with those found in healthy volunteers. There appeared to be linearity with dose for the maximum concentration of LDP-02 and area under the curve measured after IV administration. The clearance and the terminal elimination half life appeared to be independent of IV dose administration. The volume of distribution appeared to decrease slightly with

increasing doses of IV LDP-02. The percent of baseline MESF declines to ~10% rapidly after SC and IV administration of LDP-02 with duration of effect dependent upon dose. For the 0.15 mg/kg IV and SC dose groups, percent of baseline MESF started returning to baseline approximately 10 days after dosing whereas this started to occur at ~30 days and ~60 days for the 0.5 mg/kg IV and 2.0 mg/kg dose groups, respectively.

Appendix to Study L297-006

LDP-02 Serum Concentration Over Time by Subject by Treatment Group. Data obtained from individual subjects are presented in Tables 26-30. The data presented in Tables 26-30 are in μg/mL.

Table 26 Group 1: 0.15 mg/kg LDP-02 SC

Time (day)	Subject # 201101	Subject # 301103	Subject # 302105	Subject # 304107	Subject # 401104
Pre-Dose	BQL	BQL	BQL	BQL	BQL
0.125	BQL	0.07	BQL	BQL	NS
2	0.61	0.91	0.94	1.01	1.29
3	0.90	1.10	1.29	1.49	1.65
5	0.76	1.48	NR	1.66	1.74
10	0.15	1.12	1.40	0.92	1.44
14	BQL	0.61	0.78	0.24	0.99
21	BQL	BQL	NS	0.11	0.65
30	BQL	0.33	0.84	0.26	0.12
60	BQL	0.23	0.37	0.30	BQL

BQL= reported as non-detectable

NS= no sample received from laboratory

Table 27 Group 2: 0.15 mg/kg LDP-02 IV

Time (Day)	Subject # 101201	Subject # 102202	Subject # 305204	Subject # 402203	Subject # 403206
Pre-Dose	BQL	BQL	BQL	BQL	BQL
0.125	4.14	4.88	3.35	2.34	3.30
2	NR	2.74	1.92	1.83	2.34
3	3.12	3.15	1.55	1.42	2.03
5	1.82	1.83	1.33	0.82	1.19
10	0.81	0.88	0.86	0.37	0.79
14	0.32	0.15	BQL	0.23	0.26
21	0.38	0.12	0.10	BQL	BQL
30	0.38	BQL	0.40	BQL	0.05
60	0.24	BQL	0.36	BQL	0.14

NR= no sample result reported from laboratory

Table 28 Group 3: 0.5 mg/kg LDP-02 IV

Time (day)	Subject # 206302	Subject # 208303	Subject # 309306	Subject # 502304	Subject # 503307
Pre-Dose	BQL	BQL	BQL	BQL	BQL
0.125	14.06	12.33	7.90	8.67	9.76
2	10.01	8.51	5.73	5.84	8.26
3	6.56	6.45	4.96	4.67	7.27
5	4.15	5.52	3.59	2.94	5.61
10	3.17	4.46	2.81	3.11	4.21
14	2.51	0.14	2.46	1.14	3.01
21	BQL	0.17	0.14	BQL	2.04
30	BQL	0.48	BQL	0.06	1.29
60	0.41	1.73	0.10	0.28	BQL

Table 29 Group 4: 2.0 mg/kg LDP-02 IV

Time (day)	Subject # 104403	Subject # 210402	Subject # 310415	Subject # 404401	Subject # 504405	Subject # 506407
Pre-Dose	BQL	BQL	BQL	BQL	BQL	BQL
0.125	30.45	38.83	37.66	29.71	28.90	32.18
2	32.18	28.22	35.14	27.49	27.49	26.87
3	23.93	17.40	27.49	24.45	22.92	22.46
5	21.52	15.34	21.52	18.42	21.52	17.79
10	13.10	41.11	14.82	13.10	10.99	11.96
14	11.72	3.13	13.10	11.23	1.22	9.03
21	7.53	0.08	10.99	8.55	0.12	5.70
30	5.80	BQL	8.26	7.02	NR	4.19
60	1.71	0.41	2.24	1.95	NR	0.06

Table 30 placebo group

Time (day)	Subject # 202102	Subject # 303106	Subject # 103205	Subject # 306207	Subject # 308305	Subject # 501301	Subject # 209404	Subject # 505406
Pre-Dose	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
0.125	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
2	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
3	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
5	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
10	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
14	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
21	BQL	BQL	NR	BQL	BQL	BQL	BQL	BQL
30	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
60	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL

BQL = below quantitation limit.

Mean Pharmacokinetic Parameters by Treatment Group. Data obtained from individual subjects are presented in Tables 31-34.

Table 31 Group 1: 0.15 mg/kg LDP-02 SC

Subject	C_{max} (μg/mL)	t_{max} (days)	$t_{½z}$ (days)	AUC_{all} (μg·day/mL)	λ_z (1/day)	AUC (μg·day/mL)	CL (mL/day/kg)	V_z (mL/kg)
201101	0.90	3	2.58	5.30	0.2692	5.86	25.61	95.15
301103	1.48	5	34.61	30.39	0.0200	41.87	3.58	178.87
302105	1.40	10	31.35	46.94	0.0221	63.68	2.36	106.55
304107	1.66	5	3.88	15.41	0.1788	16.02	9.36	52.37
401104	1.74	5	5.72	28.17	0.1212	29.16	5.14	42.45
Mean	1.436	5.6	15.628	25.242	0.1223	31.318	9.21	95.078
SD	0.329	2.607	15.921	15.813	0.1064	22.613	9.54	54.190

C_{max} = maximum concentration

t_{max} = time to maximum concentration

λ_z = a measure of elimination

$t_{1/2z}$ = terminal half-live

AUC_t = AUC_{all} = area under the curve using all time points

AUC = AUC_{ext} = area under curve extrapolated

AUC ext (%) = % of area under curve attributed to extrapolation extrapolation

V_z = apparent volume of distribution

CL = Clearance

Table 32 Group 2: 0.15 mg/kg LDP-02 IV

Subject	C_{max} (μg/mL)	t_{max} (days)	$t_{½z}$ (days)	AUC_{all} (μg·day/mL)	λ_z (1/day)	AUC (μg·day/mL)	CL (mL/day/kg)	V_z (mL/kg)
101201	4.14	0.13	54.69	39.64	0.0127	58.58	2.56	202.06
102202	4.88	0.13	3.62	25.15	0.1914	25.78	5.82	30.39
305204	3.35	0.13	19.37	34.17	0.0358	44.23	3.39	94.77
402203	2.34	0.13	4.88	12.10	0.1420	13.72	10.94	77.03
403206	3.30	0.13	11.99	23.28	0.0578	25.70	5.84	100.99
Mean	3.602	0.13	18.91	26.868	0.0879	33.602	5.71	101.05
SD	0.9579	0	20.97	10.611	0.0757	17.718	3.27	62.87

Table 33 Group 3: 0.5 mg/kg LDP-02 IV

Subject	C_{max} (μg/mL)	t_{max} (days)	$t_{½z}$ (days)	AUC_{all} (μg·day/mL)	λ_z (1/day)	AUC (μg·day/mL)	CL (mL/day/kg)	V_z (mL/kg)
206302	14.06	0.13	17.21	139.26	0.0403	149.44	3.35	83.08
208303	12.33	0.13	3.02	74.99	0.2293	75.73	6.60	28.79
309306	7.90	0.13	9.22	67.49	0.0751	68.82	7.27	96.69
502304	8.67	0.13	10.52	65.34	0.0659	69.59	7.19	109.09
503307	9.76	0.13	13.11	109.80	0.0529	134.20	3.73	70.48
Mean	10.544	0.13	10.616	91.376	0.0927	99.556	5.628	77.626
SD	2.582	0	5.229	32.207	0.0775	39.048	1.928	30.90

Table 34 Group 4: 2.0 mg/kg LDP-02 IV

Subject	C_{max} (µg/mL)	t_{max} (days)	$t_{½z}$ (days)	AUC_{all} (µg·day/mL)	λ_z (1 day)	AUC (µg·day/mL)	CL (mL/day/kg)	V_z (mL/kg)
104403	32.18	2.00	17.92	510.32	0.0387	554.52	3.61	93.22
310415	37.66	0.13	16.72	626.06	0.0415	680.08	2.94	70.92
404401	29.71	0.13	18.34	525.63	0.0378	577.22	3.46	91.68
506407	32.18	0.13	7.02	398.45	0.0988	399.06	5.01	50.75
Mean	32.933	0.13	15.0	515.12	0.0542	552.72	3.755	76.643
SD	3.360	0.935	5.364	93.19	0.0298	116.10	0.885	20.034

Serum α4β7 Binding Over Time by Subject by Treatment Group. Data obtained from individual subjects are presented in Tables 35-40. For each subject the time of blood sampling, MESF of the sample and % of baseline (pre-dose) MESF is presented.

Table 35 Group 1: 0.15 mg/kg LDP-02 SC

Time Days	Subject # 201101		Subject # 301103		Subject # 302105		Subject # 304107		Subject # 401104		Mean	
Pre-Dose	10046	100%	7326	100%	12684	100%	13117	100%	3369	100%	9308	100%
0.125	951	9%	762	10%	1700	13%	857	7%	1105	33%	1075	12%
3	797	8%	383	5%	707	6%	853	7%	575	17%	663	17%
5	845	8%	723	10%			815	6%	1052	31%	859	9%
10	675	7%	717	10%	862	7%	865	7%	941	28%	812	9%
14	4197	42%	754	10%	830	7%	905	7%	1058	31%	1549	17%
21	9610	96%	803	11%	834	7%	3443	26%	948	28%	3128	34%
30	9462	94%	1142	16%	1275	10%	1587	12%	1113	33%	2916	31%
60	9839	98%	752	10%	849	7%	1262	10%	2849	85%	3110	33%

Table 36 Group 2: 0.15 mg/kg LDP-02 IV

Time Days	Subject # 101201		Subject # 102202		Subject # 305204		Subject # 402203		Subject # 403206		Mean	
Pre-Dose	2588	100%	2712	100%	8394	100%	10016	100%	8342	100%	6410	100%
0.125	701	27%	827	30%	848	10%	642	6%	875	10%	779	12%
3	760	29%	784	29%	820	10%	679	7%	875	10%	784	12%
5	677	26%	884	33%	1012	12%	639	6%	859	10%	814	13%
10	671	26%	753	28%	943	11%	690	7%	856	10%	783	12%
14	1008	39%	1515	56%	1377	16%	608	6%	744	9%	1050	16%
21	953	37%	4220	156%	1860	22%	2044	20%	1606	19%	2137	33%
30	988	38%	328	12%	2332	28%	3302	33%	2560	31%	1902	30%
60	1680	65%	3670	135%	3275	39%	6851	68%	1168	14%	3329	52%

Table 37 Group 3: 0.5 mg/kg LDP-02 IV

Time Days	Subject # 206302		Subject # 208303		Subject # 309306		Subject # 502304		Subject # 503307		Mean	
Pre-Dose	3830	100%	11267	100%	5084	100%	5615	100%	9400	100%	7039	100%
0.125	1322	35%	1577	14%	887	17%	879	16%	1021	11%	1137	16%
3	1189	31%	2012	18%	914	18%	775	14%	982	10%	1174	17%
5	1054	28%	1717	15%	962	19%	809	14%	1147	12%	1138	16%
10	1195	31%	2108	19%	965	19%	829	15%	732	8%	1166	17%
14	1339	35%	2405	21%	1106	22%	610	11%	801	9%	1252	18%
21	1296	34%	2085	19%	671	13%	636	11%	733	8%	1084	15%
30	1483	39%	1706	15%	1203	24%	860	15%	611	7%	1173	17%
60	985	26%	1038	9%	1611	32%	764	14%	7611	81%	2402	34%

Table 38 Group 4: 2.0 mg/kg LDP-02 IV*

Time Days	Subject # 104403		Subject # 210402		Subject # 310415		Subject # 404401		Subject # 506407		Mean	
Pre-Dose	6714	100%	5026	100%	4642	100%	4235	100%	7418	100%	5607	100%
0.125	695	10%	666	13%	736	16%	671	16%	738	10%	701	13%
3	659	10%	671	13%	632	14%	760	18%	683	9%	681	12%
5	633	9%	659	13%	663	14%	730	17%	665	9%	670	12%
10	703	10%	636	13%	556	12%	778	18%	734	10%	681	12%
14	681	10%	590	12%	640	14%	658	16%	755	10%	665	12%
21	528	8%	621	12%	568	12%	586	14%	756	10%	612	11%
30	639	10%	1218	24%	599	13%	682	16%	740	10%	776	14%
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*No data for Subject # 505405

Table 39 Placebo Group

Time Days	Subject # 202102		Subject # 303106		Subject # 103205		Subject # 306207		Subject # 308305		Subject # 501301	
Pre-Dose	7657	100%	21074	100%	4935	100%	8070	100%	15162	100%	5274	100%
0.125	5643	74%	23312	111%	4935	100%	6837	85%	15162	100%	6424	122%
3	8831	115%	19528	93%	4593	93%	7162	89%	13876	92%	6022	114%
5	7158	93%	16567	79%	4452	90%	5044	63%	13094	86%	5530	105%
10	7413	97%	17575	83%	5499	111%	4750	59%	14531	96%	8201	155%
14	6092	80%	17827	85%	3222	65%	4169	52%	10294	68%	6740	128%
21	8463	111%	18048	86%			4491	56%	12700	84%	7205	137%
30	7353	96%	15817	75%	2317	47%	11458	142%	9328	62%	5745	109%
60	3385	44%	11810	56%			4771	59%	9648	64%	3262	62%

Table 40 Placebo group

Time Days	Subject # 209404		Subject # 505406		Mean	
Pre-Dose	11012	100%	7579	100%	10095	100%
0.125	11826	107%	9025	119%	10396	103%
3	10549	96%	8792	116%	9919	98%
5	11614	105%	6217	82%	8710	86%
10	8238	75%	7150	94%	9170	91%
14	8382	76%	4787	63%	7689	76%
21	7031	64%	7160	94%	9300	92%
30	6817	62%	8166	108%	8375	83%
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While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.